

Conference on Spontaneous Regression of Cancer

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Conference on Spontaneous Regression of Cancer

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NATIONAL CANCER INSTITUTE MONOGRAPHS

FRANK J. RAUSCHER, JR., *Director, National Cancer Institute*

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CONFERENCE ON SPONTANEOUS REGRESSION OF CANCER

Held at
The Johns Hopkins Medical Institutions
Baltimore, Maryland
May 9 and 10, 1974

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American Cancer Society

Edited by

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The Johns Hopkins Hospital and
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Baltimore, Maryland 21205

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INTRODUCTORY REMARKS

On behalf of the Johns Hopkins Medical Institutions and the American Cancer Society, which has sponsored this Conference, it is my genuine and heartfelt pleasure to welcome this large and distinguished audience to this, the very first, conference to be held on spontaneous regression of cancer.

For many years, physicians and surgeons alike have observed the dramatic but rare regression of an occasional case of cancer in the absence of adequate, recognized, or conventional treatment. However, only recently has the concept of spontaneous regression of cancer become respectable. Whereas the prospect is indeed exciting and intriguing, it must be emphasized that it is still, for the most part, a "triumph of hope over experience."

In this Conference we will be exploring these experiments of nature. Our search may be called a symposium of suppositions based upon old clinical observations combined with a new audacity in thinking.

I must urge the members of the press, whom we cordially welcome, to be especially cautious; for despite our own personal fervor and enthusiasm, the public *and particularly the cancer patients* must be protected from the heartbreaking frustration and disappointment of sensational claims but unfulfilled expectations. Medical research and clinical investigation have yet to provide us with the "sacrament of confirmation." Cancer is curable by early detection and diagnosis and the present, standard, accepted methods of treatment, namely surgery, radiotherapy, hormone therapy, and chemotherapy. Despite our halcyon hopes and wishful thinking, let no patient delay or procrastinate and thus jeopardize his or her prognosis with the tantalizing thought of spontaneous regression.

The purpose of this Conference on Spontaneous Regression of Cancer is to listen more closely and more attentively to those "whispers of nature," whereby a rare and extraordinary patient with clinically confirmed cancer responds in an unusual and exceptional way without adequate treatment. Perhaps there is more to hear than meets the ear. With the power of faith in the newest medical research and the prepared perceptiveness of our audience and our program panelists, we may better discern those "whispers."

I am most grateful to the Maryland Division of the American Cancer Society and its staff for their assistance in this symposium, as well as to Dr. Russell Morgan and Dr. George Zuidema for their encouragement to hold this conference at The Johns Hopkins Medical Institutions. I am deeply indebted to the members of the Program Committee for their time, knowledge, and special effort in selecting this internationally renowned and distinguished faculty. Finally, I offer my appreciation to my secretary Bertha Richards, to Maria Heyssel, and to the coordinator of this Conference. And I thank the faculty and the audience for their valuable participation in this program.

Edward F. Lewison, M.D.
Associate Professor of Surgery
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Baltimore, Maryland 21205

INTRODUCTORY REMARKS

Like Dr. Lewison, I would like to extend warm greetings to you at this first Conference on Spontaneous Regression of Cancer. This program comes at a very significant time for us. Not only are we as an Institution embarking on a new era in terms of our relationships beyond the shores of the United States, but within a few weeks we shall start also the construction of a major new cancer facility at Johns Hopkins, a comprehensive cancer center that should provide the State of Maryland and the surrounding areas with research, teaching, and treatment facilities unequalled in the past and that will bring to The Johns Hopkins University a great move forward in our attack on cancer. The buildings on the site of the new center have just been demolished; within a few weeks we shall have the ground-breaking ceremony; and sometime in 1976 the new center will be in operation.

Thus your presence here has a significant and timely relationship with our new developments in the field of cancer. Thank you for coming. We are delighted to have you, and we wish you a successful meeting.

Russell H. Morgan, M.D.
Vice President for Health Divisions
Dean of the Medical Faculty
The Johns Hopkins University
Baltimore, Maryland 21205

INTRODUCTORY REMARKS

On behalf of the American Cancer Society, Maryland Division, I want to welcome each of you to this meeting. The American Cancer Society has played a great role in many ways, we believe, toward the understanding and treatment of cancer. Obviously, we still have not reached the point of understanding at which we can sit back and offer cures. We hope that day will not be too distant; until then, we must all roll up our sleeves and continue to work toward it. In the American Cancer Society, our efforts are directed toward this end by supporting research, by offering patient care through our service programs, and by education. In this last field, emphasis is placed on both public and professional education. A meeting such as this is one of the ways we promote professional education in cancer.

A short time ago, Dr. Edward F. Lewison proposed that the Maryland Division of the American Cancer Society support an international meeting on the subject of spontaneous regression of cancer. We realized that such a program might indeed offer some insight toward a better understanding of the disease that we are pledged to eradicate.

Dr. Lewison is an outstanding man, and we of the American Cancer Society are proud to claim him as a very willing and able volunteer, a leader in the Society both in Maryland and in the National organization. Dr. Lewison is a past president of our Division and continues as an untiring worker on our board of directors. We owe many thanks to this fine man, and we are likewise grateful to his staff and committee, all of whom worked diligently to produce this forthcoming program. It took months of planning and hours of labor to accomplish the details of efficient organization and coordination of such a meeting.

The American Cancer Society is also thankful to The Johns Hopkins Medical Institutions for providing a home for this meeting and for the able assistance given by the staff. Of course, we are equally appreciative of the many individuals who are gathered here, giving their time to present this program. Many have come a long way, some even halfway around the globe. The American Cancer Society thanks you all and thanks also the many who have taken the time from their busy schedules to come to listen and learn.

Cancer will be conquered in our lifetime. We hope that after these two days, a better understanding of this disease will be possible. Spontaneous regression implies something has happened within the body to cause the disease to cease. If we can find what that something is, we may understand what is needed to help the one in four in this very audience who may be touched by cancer. We hope that the learning taken home by each of us may give rise to more knowledge that will eventually end the world's most dreaded disease. The American Cancer Society sincerely hopes that you will be pleased with this meeting and that you enjoy your stay in our State of Maryland.

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Opening Address: Spontaneous Regression of Cancer and the Importance of Finding Its Cause¹

Warren H. Cole, M.D.²

SUMMARY—A few years ago Everson and I assembled all the examples of spontaneous regression in the world medical literature from 1900 to 1960 and added numerous cases from experiences of our friends. Our figure was 176. We excluded squamous cell carcinoma of the skin, leukemia, Hodgkin's disease, and a large number of cases that did not fulfill the prerequisites of confirmed diagnosis and no significant treatment. The four most common examples of regression were carcinoma of the kidney (31), neuroblastoma (29), malignant melanoma (19), and choriocarcinoma (19); these constituted more than half the group. We did not require that the regression be permanent because it appeared that the explanation of temporary regression would be just as important as the cause of permanent regression. There was no proven specific cause of the regression, but the following mechanisms had a possible relationship: immunologic action, elimination of carcinogens, trauma (altering the antigen-antibody relationship), hormones, irradiation, infection and/or fever, and drugs or chemicals. The most applicable of these is elimination of the carcinogen. Immunologic reactions seem to offer the best explanation, and the potential of humoral immunity is more impressive than that of cellular immunity.—*Natl Cancer Inst Monogr* 44: 5-9, 1976.

I feel highly complimented to be invited to give the opening address in this Conference. Although I have given a lot of thought to a possible explanation of the strange phenomenon of spontaneous regression of cancer, I confess I have few suggestions to make. If we are going to make progress in finding answers, I suspect we will have to reach outside the realm of accepted facts about cancer and that when we find answers, they will come in the form of explanations for regressions of individual tumors. It is possible that the explanations of regressions may differ even for patients with the same kind of cancer. In any event, I feel that when we find the answer or answers to spontaneous regression, they will be applicable to the cure of cancer.

There is still doubt in the minds of some clinicians regarding the validity of spontaneous regression, but as time goes on there appear to be fewer disbelievers. Boyd (1) is one of those who has given years of thought to the subject and is convinced the condition exists. Before speculating on various possible explanations, it would be appropriate to present some of the data that Everson and I (2) encountered during our study of the subject several years ago.

REVIEW OF DATA DERIVED FROM THE STUDY BY EVERSON AND COLE

The 176 cases collected in our study (2) were obtained from the literature (1900-66) and from experiences of

our friends. We excluded leukemias, squamous cell cancer, and lymphomas. We encountered several hundred cases in the medical literature as examples of the phenomenon, but we excluded those lacking adequate biopsy confirmation and those in which the regression possibly related to the treatment.

The term "spontaneous" is an erroneous classification because there must be a cause of the regression; the term "idiopathic" or "biologic" would be more accurate. We chose to define spontaneous regression as a partial or complete disappearance of a tumor in the absence of treatment capable of producing a regression. We chose not to require complete regression of the tumor because the cause of a partial regression may be just as important as the cause of a complete regression. An analysis of our cases revealed that the regression persisted for less than 6 months in less than 21 cases, for 6 to 12 months in 10, for 1 to 2 years in 25, for 2 to 5 years in 35, for 5 to 10 years in 35, and for 10 years or more in 22. For 28 patients, there was no statement regarding the duration of the regression..

It is no doubt significant, but by no means understood, that over half of the cases occurred in cancer of the kidney (31), neuroblastoma (29), malignant melanoma (19), and choriocarcinoma (19) (table 1). Tumors in 18 organs were represented.

TABLE 1.—*Collected cases of spontaneous regression of cancer*

Type or location of cancer	Number of cases
Hypernephroma	31
Neuroblastoma	29
Malignant melanoma	19
Choriocarcinoma	19
Bladder	13
Soft-tissue sarcoma	11
Sarcoma of bone	8
Colon and rectum	7
Ovary	7
Testis	7
Breast	6
Metastatic cancer, primary unknown	4
Uterus	4
Stomach	4
Liver	2
Larynx	1
Lung	1
Pancreas	1
Thyroid	1
Tongue	1
Total	176

¹ Presented at the Conference on Spontaneous Regression of Cancer, held at The Johns Hopkins Medical Institutions, Baltimore, Md., May 9 and 10, 1974.

² Emeritus Professor of Surgery, University of Illinois College of Medicine, Chicago, Ill. Present address: 8 W. Kensington Rd., Asheville, N.C. 28804.

POSSIBLE ETIOLOGIC FACTORS IN OUR SERIES OF 176 CASES

Immunologic Factors

The most dramatic regression was encountered in 3 patients with malignant melanoma. In one instance, the regression occurred after a transfusion of blood from a patient who had developed a regression spontaneously; in another, the regression followed a blood transfusion from a patient who had sustained a 10-year cure after a radical excision of a melanoma; and in the third case, the regression followed three transfusions from the blood bank, the last of which was blood group A, Rh negative, which caused a mild reaction.

Elimination of a Carcinogen

One would expect that removal of a carcinogen from a patient's environment might account for many examples of spontaneous regression. However, we found only 10 patients who fit this category. All were patients who had a simultaneous transplant of each ureter into the colon or ileum, preliminary to cystectomy for a bladder carcinoma.

At the present time we have few examples of cancer caused by carcinogens, e.g., cancer of the lung by cigarette tar and cancer of the bladder by dyes. Since we know the carcinogenic agent causing the cancer in so few tumors, it is possible that a patient might by chance avoid the causative carcinogen and thus allow a regression to occur. It is granted that stopping cigarette smoking will not cause a lung cancer to regress, but the effect of some carcinogens may be reversible.

Body Response to Operative Trauma

The factor most frequently associated with regression was operative trauma. If there is more than a coincidental relationship, I believe that operative trauma invokes a reaction that increases the patient's immunologic resistance to his tumor.

In 43 patients regression followed excision of the primary cancer. In 22 of these patients, the presumptive pulmonary metastases disappeared after removal of the kidney with the primary cancer. In 5 additional patients, the pulmonary metastases developed after the nephrectomy, then regressed. Why should 65% of the patients having regression after excision of the primary cancer have kidney lesions? This is too high a percentage to be explained as a coincidence.

Hormone Factors

Numerous hormones, particularly the androgens and estrogens, produce regression of certain tumors, such as breast and prostate. There are innumerable examples of hormone response in our therapeutic armamentarium, i.e., enough to suggest that a large percentage of spontaneous regressions would be hormonal in origin, but there is really little evidence to support this. However, in our series, 5 ovarian carcinomatous metastases regressed after removal of one or both ovaries. Metastases from 1 sarcoma and 3 melanomas regressed after delivery of a child. Eleven other regressions appeared to have hormonal relationships.

Role of Irradiation

Regressions following irradiation were so extremely varied that it is difficult to draw conclusions. In 1 patient with a neuroblastoma, 7 X-ray treatments were given to an abdominal mass that did not respond. After this, 2 or 3 new

metastases developed. Three years later, though all masses were present, they had decreased in size by one-third. It is possible that the regression was not related to the irradiation because such a long time intervened between the treatment and the regression. Another patient had an orchiectomy for a round cell carcinoma. Shortly after this, he developed severe abdominal pain and numerous presumptive pulmonary metastases (on X-ray). He had irradiation to the abdomen, which relieved his pain. A chest X-ray 10 days later revealed no trace of the pulmonary metastases; a year later the lungs were still clear.

Infection and/or Fever

Although infection and/or fever was closely related to the regression in only 8 of the 176 cases, it was dramatic in 2 or 3. For example, a woman had a lesion of the left arm removed; a diagnosis of melanosis was made by histologic examination. Six months later an attempt was made to remove an axillary mass associated with an abscess, but excision was only partial because of the abscess. The histologic diagnosis on the axillary mass was melanosis. The scars healed, the mass disappeared, and the patient was known to be alive and well 20 years later.

Drugs

It is assumed that anticancer drugs are not curative, but we found three instances of regression possibly caused by these drugs. Two of these could represent examples of unusual sensitivity of the tumor to the drug, and the third case (choriocarcinoma treated with chlortetracycline) may not represent a drug effect because at the time the drug was stopped the patient was having symptoms from the tumor. Nine months later she improved markedly after an attack of hemoptysis; shortly thereafter she made a complete recovery.

Miscellaneous Factors

Interference with the vascular supply to the tumor resulting from an unsuccessful attempt to remove it might result in destruction of the tumor, but it would have no effect on the metastases. Severe malnutrition was encountered in 8 of our cases, but this complication would not appear to be a logical explanation because it occurs so often in patients with terminal disease.

VALID FACTS AND DATA INDIRECTLY RELATED TO SPONTANEOUS REGRESSION OF CANCER

It has long been agreed that growth or regression of a tumor depends on host resistance and virulence of the tumor. The more I observe the growth of tumors, the more apparent it is that host resistance is more important than virulence of the tumor.

Most authorities have assumed that if a tumor is going to recur after excision of the primary and obvious secondary cancers, it should do so within 2 or 3 years after the operation. It is granted that disturbance of blood supply incident to an operation might in itself slow down the metastatic growth, but most of the recurrences develop in areas distant from the operative site. On many occasions 10-15 years elapse before recurrences develop. Therefore, it appears logical that many or perhaps the majority of patients have enough resistance to delay the development of metastases for quite some time, but not enough to destroy all the tumor. There is a growing belief that immunologic procedures can destroy a few cells but not a

lot. If this is true, there would be stronger indication to resort to immunologic therapy early after operation rather than late when metastases are present.

Hormones

We have numerous examples of the beneficial effect of hormone therapy or ablation on human tumors, but we are not willing to concede that hormone therapy results in cure. Male, and occasionally female, hormones will cause a regression of 4–24 months in 25–30% of all patients with carcinoma of the breast. Also, adrenalectomy or excision of the pituitary gland will produce a regression of a few to several months in patients with breast cancer. Likewise, 25–30% of patients with prostate cancer will sustain a similar regression after orchiectomy or female hormone therapy.

Immunoglobulins

The immunoglobulin content of blood, malignant tissue, benign tissue, etc. may be related indirectly to an immunologic process. Witz (3) analyzed the tissue of primary benzo[*a*]pyrene-induced sarcoma in animals and discovered that it contained fully 10 times more immunoglobulin than did normal tissue. He also noted that higher IgG₂ fractions were obtained from fast-growing tumors and from the primary tumor rather than from the secondary cancers. This led him to believe that tumor-associated IgG₂ may actually be a specific antibody with activity against tumor cells or their function. Takasugi and Hildemann (4) found that hyperimmune serum produced in host mice against allogenic tumor antigens inhibited and enhanced tumor growth. When the antiserum was fractionated by column chromatography, they noted that IgM was chiefly “responsible for inhibition, and immunoglobulin G₂ for enhancement.” Sjögren and Bansal (5) concluded that the unblocking agents studied by the Hellströms (6) are immunoglobulins.

Roberts and associates (7) found that IgA and IgG levels were significantly lower in malignant breast tissue, whereas IgM was significantly higher. Romsdahl and associates (8) reported identification of a blocking immunoglobulin in human sarcomas.

The Immune Reaction

The immunologic response in the animal or human being can be either humoral (antibody) or cellular (lymphatic, etc.). Klein and Oettgen (9) stated that there is increasing evidence that primary tumors evoke both antibody- and cell-mediated immune reaction in the host. As would be expected, the evidence is building up faster for animals than for the human body.

Perhaps the first evidence of antibody reaction in animals was presented by Gross (10) when he reported the regression of intradermal sarcoma by autoantibody developed after injection of diluted doses of sarcoma cells. A few years later, Friend (11) reported the preparation of a vaccine that would prevent transmission of leukemia in mice. This was followed by the preparation of specific neutralizing antibodies by Stewart and Eddy (12) against the polyoma virus tumor. Baldwin (13) reported that atrophy and regression of implanted tumor induced active immunity in methylcholanthrene-induced tumors in inbred rats. Prehn and Main (14) reported the detection of tumor-specific antigens in methylcholanthrene-induced neoplasms of inbred mice. Hellström and Hellström (15) conducted

extensive experiments on tumor-specific transplantation antigens and noted that almost all animal neoplasms contain them.

For the human being, the first encouraging evidence of an immune reaction was that of Graham and Graham (16), who reported that 12 of 48 patients with cancer of various organs had a circulating antibody to their own tumor. Most of the patients without antibody had advanced cancer. Klein (17, 18) reported that immune responses by the host against tumor-associated antigens have been demonstrated for Burkitt's lymphoma, neuroblastoma, malignant melanomas, cancer of the nasopharynx, osteogenic sarcoma, and cancer of the colon and bladder. Humphrey et al. (19) recently reported finding antibody to a breast cancer antigen (by immunodiffusion or complement fixation) in 48% of 84 patients with cancer of the breast but in only 1.5% of 206 presumably normal patients. Strange to say, antibody was also found in 34% of 96 patients with fibrocystic disease and 25% of 44 patients with fibroadenoma.

Morton and associates (20) made the important observation that after surgical removal of the tumor mass in skeletal and soft tissue sarcoma, there is a rise in titer of antitumor antibody. Also, all patients with recurrent tumor had a progressive decline in that titer as the disease advanced. This suggests that as the tumor advanced, either the patient was making less antibody or more of it was being absorbed by the tumor. Alexander and Hall (21), studying rats with chemically induced primary fibrosarcoma, found that blood-borne metastases did not develop until antibody was absent.

The immunologic defense to antigenic tumor cells is based on two major systems: 1) the formation of specific antibodies that combine with and inactivate antigens and 2) the formation of specific, immunologically reactive lymphoid cells. As Metcalf (22) stated, “to initiate an immune response antigen must be released from tumor cells and pass via the lymph or blood to the lymph nodes and spleen.” This indicates how closely integrated the humoral and cellular responses are. Metcalf stated further that “large antigenic fragments require preliminary ingestion and degradation by macrophages. Small antigens of macrophage-processed antigens then come into contact with lymphoid cells which are specifically preprogrammed to bind the antigen. Interaction of antigen with these antigen-binding cells is the critical event initiating an active immune response.”

Enzymes

Recently, a few enzymes have been described as having the potential to interfere with growth of tumors. Currie and Bagshawe (23) reported that when visible tumor cells (methylcholanthrene-induced sarcoma) are incubated with neuraminidase, the incidence of successful transplantation into syngeneic animals is increased. Simmons et al. (24) reported that neuraminidase can cause regression of established animal neoplasms by active immunotherapy. Several RNA tumor viruses contain polymerase, which can reverse the normal direction of cell life. Waalkes et al. (25) studied DNA polymerase of the Walker 256 tumor and found four distinct DNA polymerase activities. L-Asparaginase is also a tumor-inhibiting enzyme.

Another enzyme that has great potential is reverse transcriptase, which can “anticode information received in the form of ribonucleic acid into a more stable form of deoxyribonucleic acid” (26).

Miscellaneous Factors

Certain nonspecific drugs might cause regression of a tumor much like a chemotherapeutic drug does. However, trials with drugs usually result in the opposite effect, i.e., increased percentage of "takes" above that of control animals. Buinauskas and associates (27) tried 15–18 different drugs with a uniform inoculation of Walker 256 carcinoma cells, and celiotomized the rats for additional stress. Only rats receiving reserpine had lower takes than controls. Reserpine neutralized or prevented the increase in takes produced by the celiotomy. This is not the same as killing cancer cells, but at least one drug was found that prevented decreased host resistance.

Interferon is a protein substance that has the capacity to inhibit virus multiplication (28); accordingly, any destructive action would be limited to those tumors caused by viruses.

FACTORS THAT COULD HAVE A DIRECT RELATIONSHIP TO SPONTANEOUS REGRESSION OF CANCER

Immunologic Resistance

I am convinced that an increase in immunologic resistance, temporary or permanent, is the best explanation of spontaneous regression. The evidence is stronger that other possibilities (e.g., cessation of contact with a carcinogen) more often produce regression, but the potential of immunologic control appears greater. One reason is the many methods by which an immunologic change can develop. Almost all immunologists agree that the immunologic process can be either antibody or cell mediated. Klein and Oettgen (9) indicated that primary tumors evoke both types of immune response.

Antigen-Antibody Complex (Humoral)

During the past few years, several groups have attempted to treat cancer (usually advanced) with vaccines or other immunologic material. Space permits presentation of only one of these trials. Krementz and associates (29) irradiated $30\text{--}160 \times 10^6$ cancer cells with 10,000–50,000 R and injected them intradermally into 19 patients at numerous areas throughout the body. The first and only the first of the 19 had a complete disappearance of the malignant melanoma up until his death from cardiac disease 4 years later. They (30) reported using a cross-transplant, cross-transfusion (plasma and white blood cells) technique in 56 patients with melanoma and eight other types of cancer, with complete response in 3 patients with melanoma, lasting 12, 46, and 51 months.

Blocking and Unblocking Agents

It is difficult to discuss antigen-antibody complex without discussing blocking agents. Hellström et al. (31) reported that sera from patients with various types of malignant tumors block the cytotoxic effect of lymphocytes immune to the specific antigen of the respective neoplasms. They found that "blocking factors can be eluted from human tumors removed at surgery." They are of the opinion that the blocking factor may be an antigen-antibody complex, an opinion also expressed by Humphrey (32).

Cellular Immunology

The lymphocyte is probably the most important of the various cellular elements involved in the immunologic process (33). Klein and Sjögren (34) were among the

first to show that lymphocytes were active against tumor cells. Nadler and Moore (35) reported infusions of large quantities of either autochthonous or allogeneic lymphocytes into 31 patients with advanced cancer, of whom 2 had temporary remissions.

Hellström and associates (36) studied blood lymphocytes in 373 patients with tumors of various kinds. "Evidence indicating antigenic cross-reactivity between tumors of the same histological type were obtained for the following seven groups of neoplasm: malignant melanoma, cancer of the colon, breast, testis, endometrium, ovary and various sarcomas." Hellström et al. (37) also noted that lymphocytes from patients with neuroblastoma were inhibitory to the growth of neuroblastoma cells.

Histiocytes have also been identified as being phagocytic for tumor cells. Draz and associates (38) noted that histiocytes from isolated guinea pig liver and spleen were capable of removing tumor cells from perfusate containing tumor cells. Likewise, histiocytes from tumor-immunized guinea pigs revealed an increase in histiocyte tumor phagocytosis in the liver and spleen.

Alteration of Antigen and/or Enhancement of Immune Response to Tumor Antigen

There are innumerable ways by which the immunologic response might be developed, but I will mention only the two methods that have been used in human beings.

Injection of BCG vaccine.—For many years, Freund's adjuvant (a bacterial product) has been known to be an effective stimulant of antibody production. In 1961, Old et al. (39) reported nonspecific stimulation of the immune system of mice by inoculation with BCG. Morton and Eilber (40) injected BCG into 8 patients with malignant melanoma; the skin tumors regressed or disappeared in 5 of them. Yashphe (41) reported that a methanol extraction residue of BCG inhibited malignant tumors in animals. Bansal and Sjögren (42) reported complete regression in 7 of 7 and 5 of 16 rats in 2 different experiments in which unblocking tumor sera combined with BCG were used.

Infection.—Many clinicians believe that Coley's toxin (made from the streptococcus), which produces severe manifestation of infection, was truly responsible for the regressions reported by Coley (43). Others are not willing to concede that. Christensen (44) reported that when rabbits with Brown-Pearce carcinoma were subjected to mass infections with hemolytic streptococcus, they did not develop metastases, whereas 50% of control animals did.

Elimination of a Carcinogen

That bladder tumors disappeared after bilateral transplantation of the ureters into the bowel suggests that something in the urine produced the tumor. Although certain chemicals might act as a causative agent, it is also likely that either a carcinogen or a virus was the causative factor.

Viral Antagonism or Interference

The presence of viral antagonism equal in degree to bacterial antagonism seen in bacterial antibiotics would not be surprising. Obviously, if this antagonism is to be effective, the tumor must be viral in origin, or viral and carcinogenic. This combined relationship in the causation of tumors is unproved, but I believe it exists. For example, Bergs (45) reported that he inoculated Sprague-Dawley rats

ip with a mixture of Moloney leukemia virus and a rat virus (strain RV-13 or GHVOB). These rats developed leukemia with less than half the frequency of the animals receiving the Moloney leukemia virus alone. He considered this to be a virus interference rather than inhibition or inactivation.

In experiments by Taylor and associates (46), mice with sarcoma-1 and Ehrlich ascites cancer were treated with bovine enterovirus-1; there was regression of the tumors without any damaging effect to the animals. They believe that the oncolytic activity is related to specific absorption of virus to the tumor cells.

Extensive trials have been reported by Wheelock and Dingle (47), who administered numerous viruses (Sendai, Newcastle disease, influenza A and B, Semlike Forest, and Sindbis) to a patient with myelogenous leukemia, described as moribund. They noted favorable effects after inoculation of each virus and concluded the consistent benefit was beyond the possibility of chance. They thought that the viruses stimulated some type of protective mechanism. This raises the question of whether the viruses produce restoration of a protective function destroyed or damaged by leukemia or stimulate a new type of protective mechanism.

REFERENCES

- (1) BOYD W: The spontaneous regression of cancer. *Can Cancer Conf* 2:354-360, 1957
- (2) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966
- (3) WITZ IP: Tumor-associated immunoglobulins. *Isr J Med Sci* 7:230-238, 1971
- (4) TAKASUGI M, HILDEMAN WH: Regulation of immunity toward allogeneic tumors in mice. I. Effect of antiserum fractions of tumor growth. *J Natl Cancer Inst* 43:843-856, 1969
- (5) SJÖGREN HO, BANSAL SC: Cited in Hellström KE, Hellström I: Cellular immunity against tumor antigens. *Adv Cancer Res* 12:167-216, 1969
- (6) HELLSTRÖM KE, HELLSTRÖM I: Some aspects of the immune defense against cancer. I. In vitro studies on animal tumors. *Cancer* 28:1266-1268, 1971
- (7) ROBERTS MM, BASE EM, WALLACE IW, et al: Local immunoglobulin production in breast cancer. *Cancer* 27:269-275, 1973
- (8) ROMSDAHL MM, COX I, NELSON RS: Identification of a blocking immunoglobulin in human sarcomas. *Proc Am Assoc Cancer Res* 19:1-2, 1974
- (9) KLEIN G, OETTGEN HF: Immunologic factors involved in the growth of primary tumors in human or animal hosts. *Cancer Res* 29:1741-1746, 1969
- (10) GROSS L: Intradermal immunization of C3H mice against sarcoma that originated in animal of same line. *Cancer Res* 3:326-333, 1943
- (11) FRIEND C: Immunological relationships of a filterable agent causing a leukemia in adult mice. I. The neutralization of infectivity by specific antiserum. *J Exp Med* 109:217-228, 1959
- (12) STEWART SE, EDDY BE: Tumor induction by SE polyoma virus and the inhibition of tumors by specific neutralizing antibodies. *Am J Public Health* 49:1493-1496, 1959
- (13) BALDWIN RW: Immunity to methylcholanthrene-induced tumours in inbred rats following atrophy and regression of the implanted tumours. *Br J Cancer* 9:652-657, 1955
- (14) PREHN RT, MAIN JM: Immunity to methylcholanthrene-induced sarcomas. *J Natl Cancer Inst* 18:769-778, 1957
- (15) HELLSTRÖM KE, HELLSTRÖM I: Cellular immunity against tumor antigens. *Adv Cancer Res* 12:167-221, 1969
- (16) GRAHAM JB, GRAHAM RM: Antibodies elicited by cancer in patients. *Cancer* 8:409-416, 1955
- (17) KLEIN G: Experimental studies in tumor immunology. *Fed Proc* 28:1739-1753, 1969
- (18) —: Immunological factors affecting tumour growth. *Br Med J* 4:418-424, 1970
- (19) HUMPHREY LJ, ESTES NC, MORSE PA, et al: Serum antibody in patients with breast disease. *Cancer* 34 (suppl): 1516-1520, 1974
- (20) MORTON DL, EILBER FR, JOSEPH WL, et al: Immunological factors in human sarcomas and melanomas: A rational basis for immunotherapy. *Ann Surg* 172:740-749, 1970
- (21) ALEXANDER P, HALL JG: The role of immunoblasts in host resistance and immunotherapy of primary sarcomata. *Adv Cancer Res* 13:1-37, 1970
- (22) METCALF D: Initiation of immune responses against cancer cells. *CA* 22:308-310, 1972
- (23) CURRIE GA, BAGSHAW KD: Tumour specific immunogenicity of methylcholanthrene-induced sarcoma cells after incubation in neuraminidase. *Br J Cancer* 23:141-149, 1969
- (24) SIMMONS RL, RIOS A, RAY PR, et al: Effect of neuraminidase on growth of a 3-methylcholanthrene-induced fibrosarcoma in normal and immune-suppressed syngeneic mice. *J Natl Cancer Inst* 47:1087-1094, 1971
- (25) WAALKES TP, SANDERS K, SMITH RG, et al: DNA polymerases of Walker 256 carcinosarcoma. *Cancer Res* 34:385-391, 1974
- (26) MCKHANN CF: Tumors. *Surg Gynecol Obstet* 134:283-287, 1972
- (27) BUINAUSKAS P, McDONALD GV, COLE WH: Role of operative stress on the resistance of the experimental animal to inoculated cancer cells. *Ann Surg* 148:642-648, 1958
- (28) WAGNER RR: Interferons considered as cell-mediated inhibitors of viral replication. In *Viruses Inducing Cancer* (Burdette WG, ed.). Salt Lake City, Univ Utah Press, 1966
- (29) KREMENTZ ET, SAMUELS MS, WALLACE JM, et al: Clinical experiences in immunotherapy of cancer. *Surg Gynecol Obstet* 133:209-217, 1971
- (30) KREMENTZ ET, MANSELL PW, HORNING MO, et al: Immunotherapy of malignant disease: The use of viable sensitized lymphocytes of transfer factor prepared from sensitized lymphocytes. *Cancer* 33:394-401, 1974
- (31) HELLSTRÖM I, SJÖGREN HO, WARNER G, et al: Blocking of cell-mediated tumor immunity by sera from patients with growing neoplasms. *Int J Cancer* 7:226-237, 1971
- (32) HUMPHREY LJ: Tumors. *Surg Gynecol Obstet* 138:220-221, 1974
- (33) PREHN RT: Cancer and the immune response. *Proc Inst Med Chic* 29:339-345, 1973
- (34) KLEIN E, SJÖGREN HO: Humoral and cellular factors in homograft and isograft immunity against sarcoma cells. *Cancer Res* 20:452-461, 1960
- (35) NADLER SH, MOORE GE: Clinical immunologic study of malignant disease: Response to tumor transplants and transfer of leukocytes. *Ann Surg* 100:482-490, 1966
- (36) HELLSTRÖM I, HELLSTRÖM KE, SJÖGREN HO, et al: Demonstration of cell-mediated immunity to human neoplasms of various histological types. *Int J Cancer* 7:1-16, 1971
- (37) HELLSTRÖM I, HELLSTRÖM KE, BILL AH, et al: Studies on cellular immunity to human neuroblastoma cells. *Int J Cancer* 6:172-188, 1970
- (38) DRAZ S, BYFIELD JE, FONKALSUD EW: Hepatic and splenic histocyte stimulation on the phagocytosis of syngeneic tumor cells. *Surg Forum* 22:111-112, 1971
- (39) OLD LJ, BENACERRAF B, CLARKE DH, et al: The role of the reticuloendothelial system in the host reaction to neoplasia. *Cancer Res* 21:1281-1300, 1961
- (40) MORTON D, EILBER R: Value of BCG in the treatment of patients with malignant melanoma. *Med World News* 11:4-12, 1970
- (41) YASHPHE DJ: Immunological factors in nonspecific stimulation of host resistance to syngeneic tumors. *Isr J Med Sci* 7:90-107, 1971
- (42) BANSAL SC, SJÖGREN HO: Regression of polyoma tumor metastases by combined unblocking and BCG treatment—correlation with induced alterations in tumor immunity. *Int J Cancer* 12:179-193, 1973
- (43) COLEY WB: Endothelial myeloma or Ewing sarcoma (results of therapy, including use of Coley's erysipelas and prodigious toxins). *Am J Surg* 27:7-18, 1935
- (44) CHRISTENSEN EA: Infection and malignant tumours. I. Growth of Brown-Pearce carcinoma in rabbits treated with living or killed hemolytic streptococci. *Acta Pathol Microbiol Scand* 46:285-295, 1959
- (45) BERGS VV: Rat virus-mediated suppression of leukemia induction by Moloney virus in rats. *Cancer Res* 29:1669-1672, 1969
- (46) TAYLOR MW, CORDELL B, SONHRADA M, et al: Viruses as an aid to cancer therapy: Regression of solid and ascites tumors in rodents after treatment with bovine enterovirus. *Proc Natl Acad Sci USA* 68:836-840, 1971
- (47) WHELOCK EF, DINGLE JH: Observations on the repeated administration of viruses to a patient with acute leukemia. *N Engl J Med* 271:645-651, 1964

I.
CLINICAL DOCUMENTATION OF SPONTANEOUS REGRESSION OF CANCER

INTRODUCTION: MORNING SESSION

Pathologists by and large are skeptics, and when I was asked to come here to talk about spontaneous regression of cancer, I said, "You don't want me to come down, because I really don't believe in it." I know that there are occasional cases, but to devote a whole symposium to this seemed to me a little far-fetched; but as I thought about it, I decided that I really ought to come here to become educated and perhaps stimulate some of the speakers.

Pathologists, as I have indicated, are generally skeptical about spontaneous regression, and they are often perhaps too critical of their clinical colleagues for certain methods of cancer treatment. This hesitancy of many pathologists, who do accept many of the reported cases of spontaneous regression, was evidenced by Dr. Cole. He and Dr. Everson went over more than 1,000 cases that had been cited in the literature, and they reduced that list to about 130, if I recall correctly, that had adequate documentation. I was going to ask Dr. Cole, concerning "adequate documentation," whether he had selected one or two pathologists who he knew were primarily interested in the subject or whether he accepted the reports of other pathologists, because pathologists do make mistakes. Since I have been in the field for such a long time, members of my house staff sometimes show me a slide without telling me its year and what I had called it; 30 years later, I have changed my mind.

I am particularly interested, Dr. Cole, in the bladder cancers you mentioned, because bladder cancers are very tricky. I have seen 3 cases of cystectomies in which the cystectomy should not have been performed, because a tremendous cystitis with a lot of papillary projections had been interpreted erroneously as cancer. I think we have to be hesitant in calling a phenomenon a spontaneous regression, and we have to have, perhaps, a certain panel of pathologists who are primarily interested in finding fault with some of the diagnoses.

In any event, I think Dr. Cole and Dr. Everson have shown, and we all realize, that there are perhaps 4 of his group of tumors that are really special types of tumors and form almost 60% of the cases reported and collected in their 130-odd cases: neuroblastomas, hypernephroma or renal cell cancer, choriocarcinomas, and malignant melanomas. Spontaneous regression of the more common cancers of the gastrointestinal tract, breast, lung, pancreas, uterus, and ovary were very small in number—maybe 1 or 2 or perhaps as many as 5 cases were from these groups. So we are not talking, by and large, about the whole field of cancer; we are picking out certain types. I think we should pick out these particular cases to see whether we can get some clues; I am all for that and that is why I am here.

For our morning session, I notice that the program has been arranged so that one of these special kinds of tumor, the kidney tumor, will be discussed. The other three will be discussed this afternoon. As a skeptic, I am looking forward to hearing the evidence of spontaneous regression of these tumors and to being educated.

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A Pathologist Looks at Spontaneous Regression of Cancer¹

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SUMMARY—We are all subjected to varying amounts of mutating, potential cancer-inducing events, which are cumulative. In most instances, the accumulations are repaired or are lethal. If a viable clone survives, it must proliferate in order to become manifest and eventually to overpower the host's normal regulatory and defense mechanisms. The proliferation may be incited and sustained by cocarcinogens, hormone excess, chronic infections such as schistosomiasis, and in the case of lymphoid cells, by immune incompetence. On the other side are the protective repairing enzymes, which serve to cut out the damaged segments of DNA and repair them as fast as they can. The normal homeostatic mechanisms tend to keep cell proliferation and activity within the beneficial bounds of need. The controlling forces that cause cells to stop reproducing themselves and differentiate to perform a useful function also tend to slow or even stop the useless proliferation if all the cells can undergo maturation to a postmitotic state. There are also the protective mechanisms of immunity: Sensitized B lymphocytes tag the antigenic sites of tumor cells and activate the T-cell macrophages to destroy the tagged cells.—*Natl Cancer Inst Monogr* 44: 15–18, 1976.

We can hardly discuss the spontaneous regression of cancer until we can agree on what cancer is. Willis, in his book on pathology of tumors (1), began by defining cancer. Unfortunately, once you have reduced the concept to a definition, you have oversimplified the problem. This is illustrated by the fact that after Willis completed his definition, he spent a whole chapter defending it. Cancer goes through a developmental process: It originates in previously normal cells that gradually transform into dysplastic or atypical cells. As the neoplastic process progresses, the abnormal cells multiply, become increasingly aggressive, spread, invade and destroy adjacent tissues, and eventually the cells may metastasize to distant sites.

In the earliest stages of development there are dysplastic lesions that have long been considered as precancerous; these include leukoplakia, Bowen's disease, kraurosis of the vulva, and dysplasia of the cervix. Just how precancerous or reversible are these lesions? Mehta et al. (2) examined 3,674 Pakistani policemen who smoked or chewed tobacco. They found 205 cases of leukoplakia; 5 years later 42.4% had regressed, whether or not the patient had stopped using tobacco. Of 117 patients followed for 10 years, only 1 developed carcinoma (at age 39).

A somewhat more advanced precancerous state such as carcinoma in situ has been described in many sites preceding or accompanying carcinoma, for example, solar keratosis, erythroplasia of Queyrat, carcinoma in situ of the bronchus, cervix, endometrium, stomach, breast, and bladder, and villous adenoma of the colon. Some scientists have

mistakenly regarded these lesions as irreversible and already cancer in situ, but Auerbach et al. (3) described multiple foci of in situ change in bronchial epithelium of heavy smokers, which regressed after cessation of smoking.

In situ carcinoma has probably been most thoroughly studied in the cervix, where it may take 5–10 years before invasive lesions appear. The largest study of untreated cases was reported by Erickson (4). Of 1,077 cases, 202 were untreated or had delayed treatment, and the patients were followed at intervals (table 1). Although 45% of the cases were still in situ when examined within 6 months to 5 years after diagnosis and only 22% had regressed in this same period, after 5- to 14-year follow-ups only 15.2% remained as in situ lesions and only 11.7% had become invasive. Of 144, 88 (61.1%) had regressed and become negative 5–14 years after initial diagnosis.

Most pathologists regard invasion as the dividing line between reversible and irreversible lesions and as generally necessary for the diagnosis of cancer. Invasion does not mean surface spread down into the endocervical glands, so often seen in cervical lesions, but rather breaching of the basement membrane with invasion of the underlying stroma in the case of epithelial growths. This breaching allows for invasion of vascular structures, for spread through the lymphatics or blood vessels to distant sites, and for the formation of metastases, the sine qua non of cancer.

Although the prognosis has been somewhat less favorable when blood vessel invasion is evident on histologic sections (5, 6), vascular invasion is not equivalent to embolization or metastasis. It is true, however, that once vessels are invaded, the possible release of cells into the circulation becomes a reality; it has been shown that circulating cancer cells are found with much greater frequency than are metastases. Coman (7) reviewed the mechanisms responsible for the origin and distribution of blood-borne tumor metastases and concluded that single embolic cells or tiny clusters lodging in the capillaries were most likely to succeed in establishing secondary sites of growth.

In 1974, Gartler (8) reported a study that utilized the difference in electrophoretic mobility of glucose-6-phosphatase(s) (G-6-P) in cells of individuals of A versus B blood type as markers. In any given cell, only A or B genes are active so that only one type of G-6-P is made by a given cell. In AB individuals, there is a heterogeneous mixture of cells in which A or B type G-6-P are active so that in any given tissue of a person with AB blood type, both enzymes are found. In one case reported, there was colon cancer with metastases to the lung. Analysis of G-6-P in the primary tumor revealed a mixture of A and B cells. Analysis of separate lung metastases revealed three different types, some almost entirely A, some almost entirely B, and others mixed A and B. This indicates the possibility of single cell clones as metastases, as well as multiple cell emboli.

What is the evidence for spontaneous regression? The classic reference is the critical review published by Everson

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TABLE 1.—*Carcinoma in situ of cervix untreated or with delayed treatment*

Time untreated (yr)	Number of cases ^a	Invasive		In situ		Atypical		Negative	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
0.5-5	58	8	14	26	45	11	19	13	22
5-14	144 ^b	17	11.7	19	15.2	20	13.9	88	61.1
Total	202	25	12.4	45	22.3	15.3	31	101	50

^a From a series of 1,077 cases of carcinoma in situ of cervix (4).

^b One case after 14 years was still in situ (84-year-old patient).

(9) in 1967, in which he accepted the spontaneous regression of 176 tumors occurring in 20 different sites. Interestingly, 56% of the cases were of 4 types, i.e., renal carcinoma, neuroblastoma, choriocarcinoma, and malignant melanoma. I am puzzled that Wilms' tumor not only was not a prominent member of the group but was not even listed. Well-documented cases of total regression have been published (10), and I have seen at least one case personally.

Discussion of causes for the regression in the cases collected by Everson (9) included the effects of hormones, infections, immunity, possible interference with nutrition, and particularly in the case of regression of urinary bladder cancer, the diversion of the urinary stream. I would assume that all cases of urinary bladder cancer were papillary lesions without invasion of the bladder wall and therefore may represent preinvasive stages of carcinoma. Finally, of course, the possibility of incorrect diagnosis must also be included.

I will briefly discuss spontaneous regression in relation to immunity of host, limited growth potential of the tumor, differentiation and maturation, and mutations.

IMMUNITY OF HOST

That immunity plays a role in the growth of cancer has long been known to experimenters. The evidence for effective humoral immunity is meager. The tumor best studied (11) for antibody induction is malignant melanoma, which often produces antigen specific for melanoma cells and incites a measurable antibody response. The antibodies have cytolytic properties against the melanoma cells in tissue culture, but the documented induced regressions have occurred after transfusion of whole blood from patients who had had spontaneous regressions of melanoma. The role of humoral immunity will be discussed further by Dr. Hellström (12).

Cellular immunity would seem to be of much greater importance. For many years the test of malignancy used by research investigators has been the ability of transplanted neoplasms to grow in the recipient host and eventually to kill the animal. Such transplants were usually to other inbred animals of the same strain. When one could cross strain or species barriers, it was even more significant of autonomy. Such animals could be protected from transplants by prior exposure to antigens of the tumor. Prior radiation and immunosuppressive drugs are similarly effective in allowing transplantation of malignant tumors between unrelated individuals.

The introduction of kidney transplantation has caused a few tragedies, but it has provided some valuable inadvertent human experimental data. Metastatic bronchogenic car-

cinoza was inadvertently transplanted with a kidney and gave rise to pulmonary metastases signaling its presence (13). After removal of the tumor-bearing kidney, the residual cancer disappeared. Nine months later a second kidney was implanted with reinstitution of immunosuppression, and evidence of the previous tumor failed to appear.

Most phenomena of experimental growth of tumors has centered about transplantation immunity and thymus-dependent lymphocytes (T cells) or macrophages. Recently, Di Luzio (14) reported that in tissue culture, one T cell was the equivalent of about 1,000 B cells in its ability to destroy tumor cells, but a 1:1 ratio was even more effective. The role of antibody from sensitized lymphocytes may be to "home" onto foreign antigen-containing tumor cells, thereby activating macrophages for localized destruction. Shin et al. (15) have shown that macrophages, inactivated by freezing and thawing or by heat, are capable of preventing transplantation of mouse lymphoma to irradiated hosts in the presence of antiserum.

Human tumors in sites that have abundant lymphoid stroma have a much better prognosis: i.e., they run a much slower course. Moore and Foote (16), in a study of 1,000 patients who had radical mastectomies, found that of 52 cases of medullary carcinoma containing lymphoid stroma, only 6 (11.5%) died within 5 years; this is five times better than is seen with the usual breast carcinoma. In addition, although 22 (42.3%) had axillary metastases at operation, 11 (50%) of the patients were well after 5 years, compared with a 27% 5-year survival in other types of breast carcinoma with axillary involvement.

Similarly, favorable statistics of survival have been found in seminoma of the testis with lymphoid stroma or focal granulomatous reaction (6). Whether the lymphoid stroma is composed of predominately T or B cells or of both is not known.

LIMITED GROWTH POTENTIAL OF TUMOR

Although it has frequently been stated that cancer is a growth of autonomous cells, there is often a dependency on, and a responsiveness to, hormones, particularly in tumors of cells that are normally hormone dependent.

In humans, the suppression of thyroid tumors by exogenous thyroid and the regression of breast and prostate carcinomas by hormones do not require detailed description here, but they emphasize the function of hormones in producing regression, even though eventually the cancer claims its host.

Tumors of placental origin present a special case of genetically half "foreign" tissue which is, therefore, much more likely to incite an immune reaction, despite the fact

that the normal counterpart of this tissue usually undergoes spontaneous involution at the termination of pregnancy (17). The special effectiveness of methotrexate in this disease may be merely aiding and abetting these two processes by producing necrosis and liberating antigenic material. Its ineffectiveness against choriocarcinoma in the male may be due to the genetic compatibility of the male disease, but this is difficult to evaluate since rarely is the choriocarcinoma pure. It is usually mixed with embryonal carcinoma, and sometimes there is also teratomatous differentiation.

Rapidly progressive malignant disease in children and indolent and latent carcinomas in older patients (plus the loss of regenerative ability of tissues in old age) suggest that growth hormone may play some role in the rate of progression of malignant disease. Greenwood (18), in a study of 4 different neoplasms almost 50 years ago, failed to find evidence to support this impression. Further data is needed on this point.

It has also been generally accepted that starvation causes some slowing of tumor cell growth, but it may be equally or more detrimental to the host's normal cells. There was one study of a brain tumor that responded favorably more than once to deprivation of arginine in the diet and began growing when arginine was put back in the diet (Rogers S: Personal communication). Whether this was a specific effect or was related to the ability of arginine to increase growth hormone secretion by the pituitary is not clear.

DIFFERENTIATION AND MATURATION

Differentiation and maturation are factors in regression of some tumors, particularly those arising from embryonal cells in infants or adults. Most cancers occur in older individuals and are composed of cells that are less differentiated than normal and are associated with varying numbers or areas of cells that differentiate sufficiently to reveal the cell type and possibly the source; however, differentiation is usually limited.

There is a small, special group of tumors that arise from embryonic or poorly differentiated fetal cells in younger individuals. When these tumors remain undifferentiated, they run a rapid course to death. When they undergo maturation and differentiate, the prognosis improves. In some instances such as neuroblastoma, they can completely differentiate to ganglioneuroma and thereby become benign [table 2; (19)]. This type of maturation is most likely to occur when the tumor occurs in infants under 6 months of age. This is different from the microscopic foci in adrenal glands of so-called neuroblastoma in situ, which is reported as an incidental finding in from 1 in 39 to 1 in 500 pediatric autopsies, but which are not found in children more than 3 months of age (10). When one compares this incidence with that of overt neuroblastoma, which is about 1 in 10,000 babies, it is evident that this in situ lesion is not a tumor at

all but merely delayed maturation of medullary cells. Nor are these tumors malformations, because differentiation can take place even after metastases have occurred. Maturation may occur spontaneously, which presumably accounts for the spontaneous regressions noted in the statistics of Everson (9). Most likely maturation can also be accentuated or induced by radiation.

Wilms' tumor, capable of the same type of maturation (10), may also be accentuated by radiation. I believe the effects are reflected in the cure rates obtained in infants—rates which are dependent upon the time of initial appearance and diagnosis.

Another outstanding example of differentiation and maturation is seen in embryonal carcinoma of the testis, which by somatic differentiation converts to benign adult-type teratomatous structures. Pierce et al. (19) found that ip transplantation of foci of embryonal carcinoma in mice gave rise to numerous embryoid bodies with three germ layers. When these embryoid bodies were transplanted sc, they gave rise to teratomas. Cloning of individual embryonal carcinoma cells also revealed their multipotentiality (20). Further transplantation of differentiated tissue was unsuccessful, which shows its benign character. There is a complete parallelism in testicular tumors in man, which reveals improving prognosis proportional to the differentiation present (21).

Pierce (personal communication) more recently has shown that keratinized, differentiated portions of squamous cell carcinomas are postmitotic and cannot be transplanted. It is the associated less differentiated cells that sustain the progressive disease. Thus if one can induce maturation or differentiation in tumors, it should improve the prognosis. This seems to be aided by radiation in infants with neuroblastoma and Wilms' tumor (10).

MUTATION

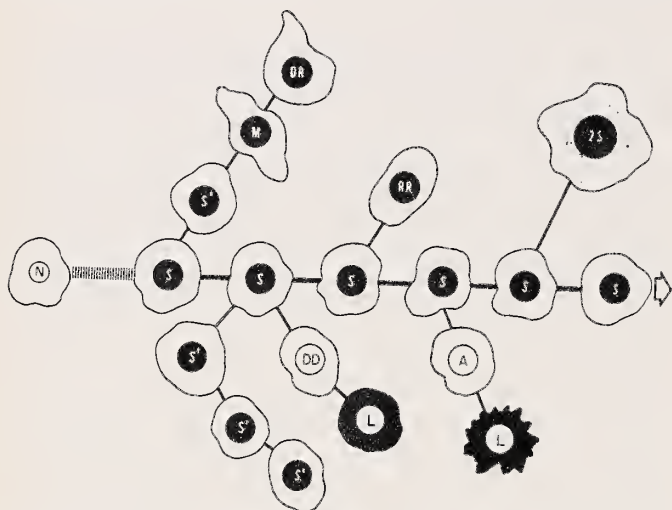
Mutation of somatic cells probably represents the initiating event or events in cancer. Whether it is manifest depends upon 1) the extent of the damage (too much damage is lethal), 2) the repair of the damage by repairing enzymes, and 3) a subsequent overgrowth by the abnormal cells and their offspring to the detriment of the normal cells and the host.

Hauschka (22) pointed out the increasing instability of cancer sublines of cells and illustrated them with a diagram (text-fig. 1). This diagram explains both the subsequent development of lethal mutations that might lead to regression and also the varying metabolic patterns that might lead to dependency because of the deletion of certain metabolic pathways and lethal effects from hormones or drugs, or their withdrawal. Radiation and some of the mutant drugs for treating cancer may, in addition to their killing action, increase the likelihood of lethal mutation developing in the unstable cancer cells.

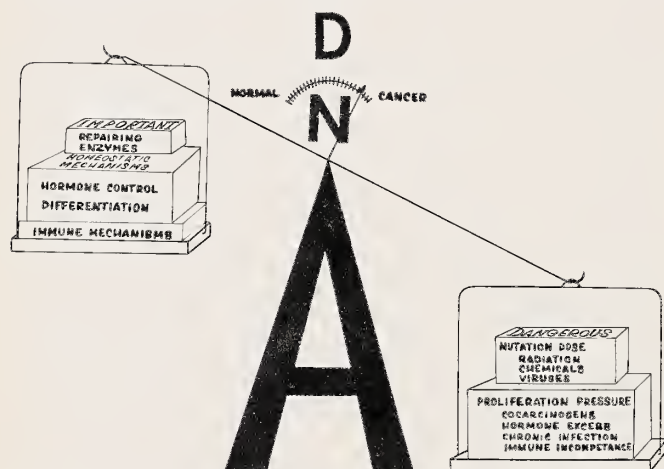
TABLE 2.—Correlation of time of initial diagnosis with survival and spontaneous regression of neuroblastoma (19)

Neuroblastoma	Number of cases	Diagnosed:				
		Neonatally	Under 6 mo	During 1st yr	During 2d yr	After 2d yr
2-year survival (%)		62-70		35-50	19	5
Spontaneous regression	29		21 (72%)			
Maturation to ganglioneuroma	16		11 (69%)			

Finally, from recent studies of unusual susceptibility to multiple skin cancers from ultraviolet irradiation in patients with xeroderma pigmentosum, it is clear that all of us are constantly bombarded with mutating events that would lead to cancer were it not for our DNA-repairing enzymes, which these unfortunate people lack. Consequently, protection against mutant radiation, chemicals, viruses, or other causative agents and an increase in the efficiency or effectiveness of the repairing mechanisms should reduce the incidence and prevent the development of cancer.



TEXT-FIGURE 1.—Diagram illustrating tumor progression through stemline variability. The nuclear symbols represent the following cell types: N, nonmalignant; S, main stemline; S¹, secondary stemline; 2S, polyploid stem cell variant; M, metastatic cell; DR, drug-resistant mutant; RR, cell with increased radiation resistance; DD, drug-dependent mutant; A, antigenic mutant; L, mutations lethal for individual tumor cells. [Reprinted from (22) with permission of the author and publisher.]



TEXT-FIGURE 2.—Diagram of known and probable factors affecting the course of cancer.

DNA is the fulcrum in the balance of forces (text-fig. 2). Which way the balance swings depends, at least in part, on the relative forces shown in text-figure 2. Once the balance is tipped in favor of cancer, can it be returned?

REFERENCES

- (1) WILLIS RA: Pathology of Tumors, 4th ed. New York, Appleton-Century-Crofts, 1967, pp 1-8
- (2) MEHTA FS, SHROFF BC, GUPTA PC, et al: Oral leukoplakia in relation to tobacco habits. A ten-year follow-up study of Bombay policeman. *Oral Surg* 34:426-433, 1972
- (3) AUERBACH O, STOUT AP, HAMMOND EC, et al: Bronchial epithelium in former smokers. *N Engl J Med* 267:119-125, 1962
- (4) ERICKSON C: Presented at American Society of Cytology when receiving Papanicolaou Award, 1965
- (5) GRAHAM A: Malignant epithelial tumors of thyroid: Invasion of blood vessels. *Surg Gynecol Obstet* 39:781-790, 1924
- (6) DIXON FJ, MOORE RA: Testicular tumors. A clinicopathological study. *Cancer* 6:427-454, 1953
- (7) COMAN DR: Mechanisms responsible for the origin and distribution of blood-borne tumor metastases: A review. *Cancer Res* 13:397-404, 1953
- (8) GARTLER S: A clonal disease. Presented at Symposium on Neoplasia and Its Origins, American Association of Pathologists and Bacteriologists, San Francisco, Calif., March 1974
- (9) EVERSON TC: Spontaneous regression of cancer. *Prog Clin Cancer* 3:79-95, 1967
- (10) BOLANDE RP: Benignity of neonatal tumors and concept of cancer regression in early life. *Am J Dis Child* 122:12-14, 1971
- (11) MORTON DL, EILBER FR, MALMGREN RA: Immune factors in human cancer: Malignant melanomas, skeletal and soft tissue sarcomas. *Prog Exp Tumor Res* 14:25-42, 1971
- (12) HELLSTRÖM KE, HELLSTRÖM I: Spontaneous tumor regression: Possible relationship to in vitro parameters of tumor immunity. *Natl Cancer Inst Monogr* 44:131-134, 1976
- (13) WILSON RE, HAGER EB, HAMPERS CL, et al: Immunologic rejection of human cancer transplanted with a renal allograft. *N Engl J Med* 278:479-483, 1968
- (14) DI LUZIO NR: The role of the reticuloendothelial system and recognition factors in neoplasia. Presented at Symposium on the Reticuloendothelial System: Function, Reaction, Neoplasia. Meeting of International Academy of Pathology, San Francisco, Calif., March 1974
- (15) SHIN HS, HAYDEN ML, GATELY CL: Antibody-mediated suppression of lymphoma: Participation of platelets, lymphocytes and nonphagocytic macrophages. *Proc Natl Acad Sci USA* 71:163-166, 1974
- (16) MOORE OS JR, FOOTE FW JR: The relatively favorable prognosis of medullary carcinoma of the breast. *Cancer* 2:635-642, 1949
- (17) BARDAWIL WA, TOY BL: The natural history of choriocarcinoma: Problems of immunity and spontaneous regression. *Ann NY Acad Sci* 80:197-257, 1959
- (18) GREENWOOD M: Natural Duration of Cancer. (Forms No. 33, Rep Publ Health, M Subj, Ministry of Health Report). London, H M Stat Off, 1926
- (19) PIERCE, GB, DIXON FJ JR, VERNEY EL: Teratocarcinogenic and tissue-forming potentials of the cell types comprising neoplastic embryoid bodies. *Lab Invest* 9:583-602, 1960
- (20) KLEINSMITH LJ, PIERCE GB JR: Multipotentiality of single embryonal carcinoma cells. *Cancer Res* 24:1544-1552, 1964
- (21) MOSTOFI FK, PRICE EB: Tumors of the Male Genital System. Atlas of Tumor Pathology, 2d series. Washington, D.C., American Registry of Pathology, Armed Forces Institute of Pathology, 1973
- (22) HAUSCHKA TS: The chromosomes in ontogeny and oncogeny. *Cancer Res* 21:957-973, 1961

Spontaneous Regression of Genitourinary Cancers¹

H. K. A. Schirmer²

SUMMARY—In genitourinary cancers, spontaneous regression has occurred in few instances and for reasons unknown. Although it cannot be denied that nephrectomy may have promoted regression of distant metastases in some cases, this is an unproved relationship and, in any event, an extremely rare sequence.—*Natl Cancer Inst Monogr* 44: 19, 1976.

Spontaneous regression of cancer has been defined as the partial or complete disappearance of a malignant tumor in the absence of all treatment or in the presence of therapy that is considered inadequate to exert a significant influence on neoplastic disease (1). There are several types of spontaneous regression. Various neoplastic tumors may cease growing and actually become smaller to a degree, only to resume their proliferation again at a later date; or any or all of the new growth may be completely absorbed, and the patient apparently cured.

Modalities such as adequate surgery, cryosurgery, laser beam, irradiation, chemotherapy, and isotope therapy do not in themselves manifest a spontaneous regression. Consequently, in the restricted sense, only spontaneous regression of genitourinary cancers following therapy considered inadequate to exert significant influence on the neoplastic disease should be reported. A search for such cases was made, and the cases were categorized according to the primary histopathologic diagnosis: renal adenocarcinoma or hypernephroma, testicular teratocarcinoma, and embryonal cell carcinoma, and prostatic adenocarcinoma.

Renal adenocarcinoma or hypernephroma received most of the attention, because it has been alleged that removal of a primary renal adenocarcinoma in the presence of distant metastases can sometimes promote regression of the metastasis (2). In a critical review of 18 cases (reported by various investigators) of spontaneous regression of apparent pulmonary metastases after excision of the primary hypernephroma, Middleton (2) pointed out the absence of biopsy confirmation of pulmonary metastases in most of the cases.

Garfield and Kennedy (3), in their study on regression of pulmonary metastases following nephrectomy for hypernephroma, found that older males were the majority of patients in whom such regression was observed. In his report on spontaneous regression in renal carcinoma,

Robinson (4) concluded that regressive changes such as fibrosis, hyalinization, calcification, and ossification are not uncommon in hypernephroma, and the regression may be so extreme that only small areas of living tumor can be found even when the growth is very large.

An unusual 5-year survival in a 22-year-old male following orchiectomy for embryonal cell carcinoma with biopsy-confirmed, tumor-positive, midaortic lymph nodes was reported by Jones (5). There was no evidence of metastases on chest X-ray before orchiectomy or 5 years later. No other therapeutic modality had been applied in this case.

In other reported cases of spontaneous regression of cancers of testicular origin, the regressions lasted less than 5 years; despite the use of invariably repeated localized irradiation, the patients eventually died from cancer (6, 7).

Thus for prostatic cancers the assessment of regression of metastases following repeated cryosurgery must be coupled with such factors as the presence of a tissue-specific antigen in the human prostate, the known production of auto-antibodies to rabbit prostate tissue complex, and the booster phenomenon after repeated freezing of this complex. Gursel and co-workers (8) reported that two of five postmortem examinations on patients with metastatic prostatic cancer treated by sequential cryotherapy showed no cancer within the remainder of the prostate. Though palliation apparently was achieved in many of their cases, objective remission and diminution of apparent metastases after cryosurgery of the prostatic primary tumor was observed only in one instance. However, the concept that necrotic tumor tissue may assume antigenic powers that simulate a specific antibody response can be supported by these observations made after cryosurgery of the prostatic primary cancer.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966, pp 419-435
- (2) MIDDLETON RG: Surgery for metastatic renal cell carcinoma. *J Urol* 97:973-977, 1970
- (3) GARFIELD DH, KENNEDY BJ: Regression of metastatic renal cell carcinoma following nephrectomy. *Cancer* 30:190-196, 1972
- (4) ROBINSON CE: Spontaneous regression in renal carcinoma. *Can Med Assoc J* 100:297-300, 1969
- (5) JONES GH: Embryonal carcinoma of the testis. An unusual five-year survival. *J Urol* 96:794, 1966
- (6) MALAMENT M, JOHNSTON WW: Spontaneous regression of pulmonary metastases arising from a testicular tumor. *J Urol* 73:117-123, 1955
- (7) BIRKHEAD BM, SCOTT RM: Spontaneous regression of metastatic testicular cancer. *Cancer* 32:125-129, 1973
- (8) GURSEL E, ROBERTS M, VEENEMA RJ: Regression of prostatic cancer following sequential cryotherapy to the prostate. *J Urol* 108:928-932, 1972

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Spontaneous Regression of Colon Carcinoma¹

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SUMMARY—Apparent spontaneous regression of advanced colon carcinoma has been reported in 7 patients. This is a very rare event, and the circumstances accounting for this are elusive.—*Natl Cancer Inst Monogr* 44: 21, 1976.

In the United States and most countries of Europe, carcinoma of the colon and rectum is the most common cause of death due to cancer and, excluding skin cancer, is the most frequently occurring form in this country. Despite the frequency of this disease, Everson and Cole's (1) monograph listing 176 cases of well-documented spontaneous regression of cancer cited only 7 cases in which the colon and rectum were affected during the years 1918–60. Dr. Charles Moertel of the Mayo Clinic, who in the last decade has followed closely several thousand patients with gastrointestinal cancer, reported (personal communication) that he has not seen one spontaneous regression, although in 1950 and 1951, 2 of the 7 spontaneous regressions reported by Everson and Cole were from the Mayo Clinic.

Second-look operations, performed subsequent to primary resection as a search for unrecognized disease left behind at the time of initial surgery, have brought about a modest decreased incidence of residual cancer, probably as a result of more extended primary procedures performed between 1948 and 1964; but they have not significantly increased the 5-year survival rate for lymph node-positive cancer and have not identified patients undergoing spontaneous regression (2).

The 7 reported cases of spontaneous regression initially presented extensive disease with penetration of the bowel wall and with lymph node and/or liver metastasis. The data do not suggest any influence of age or sex of patient or anatomic site of the tumor that might have had a favorable effect. The regressions involved local recurrences after resection, primary tumor with local extension, liver metastasis, and a multiple intra-abdominal metastasis with peritoneal implants.

The clinical impression that hepatic metastasis from colon carcinoma portends a dismal outlook for long-term survival is well documented in many series, with median survival times of 5–6 months. However, approximately 10% of the patients with hepatic metastasis described by Abrams and Lerner (3) lived longer than 2 years without any form of chemotherapy or resection of the metastasis, and 1 patient

survived almost 7 years without therapy. There seems to be no unique characteristic of the rare patient that survives an inordinate amount of time.

Everson and Cole's 2 patients with hepatic metastasis were documented as having visible and palpable tumor regression. Among the 7 patients undergoing regression, there was no documentation of cellular response in the regional lymph nodes or of lymphocytic infiltration of the carcinomas, although lesions in 2 patients were associated with marked localized inflammatory reaction. The tumors were all adenocarcinomas; and possible regression attributable to potassium depletion, as may occur in a villous adenoma, cannot be evoked. It was not reported whether these patients had general immunologic hypersensitivity with associated disease such as asthma or rheumatoid arthritis. Three patients had significant chronic infections: 2 with cutaneous fistulas and abscess formation and 1 patient with a large vesicovaginal fistula. Perhaps one could speculate on this thread of evidence, and it is only a thread, that chronic inflammation exerted some active, nonspecific stimulation of the immune response. The role of necrotic tissue and invading bowel organisms remains speculative.

The fact that these patients had intra-abdominal bulky tumor masses seems to preclude a nonspecific immunotherapy effect, because this modality is most effective against occult, residual, microscopic foci and is relatively ineffective against visible tumor. There is no panel for collection of sera or even lymphocytes or tumor cells from these patients for future immunologic studies, and perhaps one should make a plea for the establishment of a National Serum Bank and a registry for patients undergoing spontaneous regressions, as Dr. Burchenal (Memorial Sloan-Kettering Cancer Center, New York, N.Y.) has done for patients with acute leukemia in remission longer than 5 years.

In colon carcinoma, the proportion of late cancer deaths is relatively small, about 5–10% of those surviving 5 years. All 7 patients with spontaneous regression of colon cancer were alive at a median period of 9 years after diagnosis (1). It is attractive to postulate that most patients were cured.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966
- (2) GRIFFIN WO, WANGENSTEEN OH: Second-look operation. In Progress in Clinical Cancer (Ariel IM, ed.). New York, Grune & Stratton, 1965, pp 1954–1959
- (3) ABRAMS MJ, LERNER HJ: Survival of patients at Pennsylvania Hospital with hepatic metastasis from carcinoma of the colon and rectum. *Dis Colon Rectum* 14:431–434, 1971

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Spontaneous Regression of Breast Cancer¹

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SUMMARY—The dramatic but rare regression of a verified case of breast cancer in the absence of adequate, accepted, or conventional treatment has been observed and documented by clinicians over the course of many years. In my practice limited to diseases of the breast, over the past 25 years I have observed 12 patients with a unique and unusual clinical course valid enough to be regarded as spontaneous regression of breast cancer. These 12 patients, with clinically confirmed breast cancer, had temporary arrest or partial remission of their disease in the absence of complete or adequate treatment. In most of these cases, spontaneous regression could not be equated ultimately with permanent cure. Three of these case histories are summarized, and patient characteristics of pertinent clinical interest in the remaining case histories are presented and discussed. Despite widespread doubt and skepticism, there is ample clinical evidence to confirm the fact that spontaneous regression of breast cancer is a rare phenomenon but is real and does occur. *Natl Cancer Inst Monogr* 44:23-26, 1976.

Breast cancer is an all too common universal tragedy, malign and monstrously destructive of human life. In the United States, breast cancer is the most common site of this disease in women, and it is the leading cause of cancer death in our female population. About 28% of all cancers in women occur in the breast, and 6% of American women, i.e., 1 woman out of every 15, will ultimately develop this dread disease.

Few aspects of modern medicine have more pitfalls for the clinically unwary than the correct appraisal and confirmed documentation of spontaneous regression in breast cancer. However, it may be of some solace and comfort to the afflicted to know that St. Agatha (fig. 1) is the patron saint of breast cancer and that St. Peregrine (fig. 2) has been referred to (1) as the patron saint of spontaneous regression (fig. 3). According to authoritative legends of the church, St. Peregrine suffered from a cancer of his leg and was advised by his surgeons that amputation was necessary. The night before his operation he faithfully prayed to be saved from cancer surgery. In his dreams he imagined that he was cured. Upon awakening, his vision was true; he was miraculously and spontaneously cured of his cancer. He lived a long and fruitful life, dying in 1346 at the age of 80 without further evidence of cancer. He was canonized in 1726 and has become the patron saint of cancer and malignant diseases.

Of particular historical interest to those of us here at The Johns Hopkins Medical Institutions is the early report of Sir William Osler (2), who in 1901 published a paper entitled "The Medical Aspects of Carcinoma of the Breast, With a Note on the Spontaneous Disappearance of Secondary Growths" (text-fig. 1). The first of 2 cases reported by Osler was that of an unmarried woman, 31 years

old, who complained of a painful lump in her right breast, which she attributed to a fall from a bicycle. The patient was operated on by Dr. Halsted in 1897, and a right radical mastectomy was performed for breast cancer with positive axillary metastases. The patient remained free of recurrence for 1 year, after which she developed back pain radiating down her legs, with metastatic involvement of the spine, right eye, and opposite breast. She soon became bedridden and, as noted by Osler, "her condition seemed really desperate." For the relief of pain the patient required 6 grains of morphine daily. Shortly thereafter Osler left for England and, of course, never expected to see her again. Upon his return, however, the condition of the patient's metastases had improved, her pain had decreased, and the tumor of the left breast and sternum had disappeared. In October 1900, 3 years after her operation and 2 years after her widespread metastatic disease, Osler notes that "she drove a mile and a half to the station to meet me, and drove me back to the station on my return."

In conclusion, Osler notes that the phenomena presented by these cases "are among the most remarkable which we witness in the practice of medicine, and illustrate the uncertainty of prognosis, and the truth of the statement that no condition, however desperate, is quite hopeless."

A review of the collected cases of spontaneous regression reported by Everson and Cole (3) in their compendium of world literature reveals a total of only 6 confirmed cases of spontaneous regression of breast cancer. In view of the well-known hormone dependence of some cases of breast cancer, the authors suggested that destruction of the ovaries, adrenals, or pituitary by metastatic involvement may be responsible for these very rare instances of spontaneous regression. Hormonal changes at the time of the menopause also has been observed to cause a temporary regression in breast cancer, and therapeutic oophorectomy has been used for many years in the treatment of advanced breast cancer.

Sir Astley Cooper (4) noted a patient whose breast cancer improved slightly at the start of each menstrual period, and Nunn (5) in 1882 described a woman whose breast cancer underwent "general shrinking" following severe emotional trauma and the sudden onset of the menopause. The favorable response to therapeutic castration in about one-third of premenopausal patients with advanced breast cancer confirms the concept that ovarian hormones have a profound effect upon the clinical course of these breast cancers, which are hormone sensitive.

William Boyd (1), a preeminent pathologist and pioneer in the field of spontaneous regression of cancer, reported a small group of patients (collected from the literature) whose metastatic breast cancer had disappeared without adequate treatment or known cause. Long-term remission in breast cancer is a striking phenomenon of biologic control, which has been noted by Morton and Morton (6), Sir Gordon Gordon-Taylor (7), and Stewart (8) in their comprehensive reviews of restrained mitotic activity for protracted periods of time. I have seen 2 patients whose breast cancers recurred 22 and 25 years after surgery. Both

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ORIGINAL ARTICLES

THE MEDICAL ASPECTS OF CARCINOMA OF THE BREAST, WITH A NOTE ON THE SPONTANEOUS DISAPPEARANCE OF SECONDARY GROWTHS.

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TEXT-FIGURE 1.—Early report by Sir William Osler on the spontaneous regression of breast cancer.

of these patients were favorable responders to hormone therapy and lived an additional 5 and 7 years, respectively.

In my practice limited to diseases of the breast, over the past 25 years I have observed 12 patients with a unique and unusual clinical course valid enough to be regarded as spontaneous regression of breast cancer. These are patients with clinically confirmed breast cancer, who underwent temporary arrest or partial remission of their disease in the absence of complete or adequate treatment by current standards. This regression must not be equated with the permanent cure of breast cancer. The salient clinical histories of 3 of these patients follow.

CASE HISTORIES

1) A 35-year-old female underwent surgery for extensive breast cancer in 1956. She remained well for 3 years. In 1959, severe back pain developed as the result of metastases to the spine, ribs, and pelvis, with collapse of the T-5 vertebra. She was treated by oophorectomy, radiation and hormone therapy, and her condition improved for the next 2 years. However, in 1961 she became a severe alcoholic, and it was necessary to discontinue all hormone therapy, and Antabuse (disulfiram) was started. Over the next 10 years (1961-71), complete resolution of all bone lesions in the spine, skull, pelvis, and ribs gradually occurred, and the patient remained clinically free of cancer with no further androgens, irradiation, or chemotherapy. Frequent psychiatric care was required, and she remained on and off Antabuse therapy for her continued drinking problem. She died in 1971 when she accidentally fell from a third-floor window. The coroner's report showed a high blood alcohol level and residual nests of metastatic carcinoma in the bone marrow.

2) A 46-year-old female had a radical mastectomy for breast cancer in 1963. Because of positive axillary metastases, the patient had a prophylactic oophorectomy. Severe low back pain was first noted in 1968. X-rays and tomograms confirmed the presence of osteolytic metastases to T-10, L-4, and L-5. The patient was treated with radiation therapy to the spine and had relief of pain. Hormone therapy was started, but this was discontinued after 1 month because of cosmetic changes and concern over virilizing signs. The patient has remained well and clinically asymptomatic from 1968 to the present with no further treatment. She plays tennis regularly without pain, although her bone X-rays continue to show evidence of metastatic disease.

3) A 57-year-old female underwent surgery in 1963 for breast cancer with axillary metastases. In 1964 she developed a cough and dyspnea and was found to have pulmonary and pleural metastases, confirmed by cyto-

pathology. She was treated with estrogens for 2 years with a marked improvement in her lung condition. In 1965 she developed lytic metastases in her femur and pelvis and was treated with radiotherapy and androgens. She continued hormone therapy for 2 years until 1968, at which time she was free of pain and her X-ray studies showed improvement. The patient then decided to discontinue her medication and move to Florida. From 1968 until 1973, the patient remained perfectly well without any anticancer treatment, and her chest X-rays and bone scans and surveys were considered normal. Ultimately, however, the patient developed further evidence of liver and osseous metastases and is now receiving radiotherapy and chemotherapy at this hospital.

PERTINENT QUESTIONS

In carefully analyzing the 12 cases of spontaneous regression that I have observed, the following "whispers of nature" appear to be of particular and pertinent interest.

1) Five of these 12 patients had a diffusely enlarged or nodular goiter. However, only 1 patient developed hyperthyroidism and she was treated medically. The other 4 patients with a thyroid enlargement were euthyroid by clinical and laboratory examinations. Is there a subtle but real relationship, deserving further investigation, between the breast and the thyroid, as suggested by epidemiologic and empiric studies?

2) One patient remained clinically free of progressive metastatic disease for 10 years while taking Antabuse. During this time she continued to drink excessively but intermittently. Antabuse interferes with the complete catabolism of alcohol, resulting in an increase in blood acetaldehyde concentration. Can this Antabuse-alcohol reaction resulting in high acetaldehyde blood levels be chemotherapeutic for breast cancer? Does an elevated immunoglobulin level, known to occur in some alcoholics, result in an enhancement of host immunity toward cancer?

3) One patient, a missionary nurse from Kenya, had her recurrent breast cancer in control for 18 years while still having periodic recurrent episodes of malaria. She is alive and clinically well at the present time. Dramatic regression of cancer has been known to follow high fever and certain bacterial infections. Can periodic flare-ups of malaria serve as a control of malignant diseases?

4) A severely psychotic male patient had a lobotomy performed about 7 years prior to the onset of breast cancer. After orchiectomy and hormone therapy for extensive lung and bone metastases, he improved dramatically and refused further medication. He remained well for 3 years without treatment and ultimately died of cardiac failure. Can a lobotomy interfere with pituitary function sufficiently to disturb the estrogen, prolactin, and growth hormone relationship in breast cancer?

CLINICAL CHARACTERISTICS OF BREAST CANCER

Finally, I would like to call your attention to several noteworthy clinical characteristics of breast cancer, which seem to be quite impressive and extraordinary. Unfortunately, these observations are for the most part little known and generally unexplained. However, they are curious common denominators of breast cancer and certainly seem worthy of further study.

1) Advanced breast cancer is often a widely disseminated disease, yet it almost never metastasizes below the elbow or below the knee.

2) Metastatic breast cancer can and often does involve every organ in the body *except one*. This one structure is the largest in the human body and consists of the voluntary or striated muscles. There are over 400 striated muscles on each side of the body, and although they may be affected by the direct invasion of cancer, they are never affected by metastatic dissemination.

3) Breast cancer is a common disease throughout the animal kingdom, frequently affecting the dog, cat, mouse, rat, and most other mammals. However, the cow with its large and highly specialized mammary gland is the only exception to this prevalent cancer. Breast cancer of epithelial origin has rarely, if ever, been found in the udder of a cow, and these domestic animals have been very carefully examined over a long period of time.

In conclusion I would like to quote Sir Gordon Gordon-Taylor (7) who said that there was a time not long ago "when the competitive spirit in surgeons exhausted itself in vain-glorious boastings of the removal of tumors remarkable for their pounds. . . . Surgical megalomania is now more happily satisfied by the recordings of unexpected, even unprecedented years of survival of patients

after operation, radiotherapy or hormonal or other form of treatment for malignant disease."

Thus clinical experience has confirmed that these incredible cases of spontaneous regression do exist. May the concepts conceived at this conference bring us nearer and nearer the ultimate control of breast cancer.

REFERENCES

- (1) BOYD W: The Spontaneous Regression of Cancer. Springfield, Ill., Charles C Thomas, 1966, pp 1-99
- (2) OSLER W: The medical aspects of carcinoma of the breast, with a note on the spontaneous disappearance of secondary growths. *Am Med* Apr 6, 1901, pp 17-19; April 13, 1901, pp 63-66
- (3) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966
- (4) COOPER A: Lectures on the Principles and Practice of Surgery (3 vol). London, Frederick Tyrrell, 1829
- (5) NUNN TW: Cancer of the Breast. London, Churchill, 1882, p 103
- (6) MORTON JJ JR, MORTON JH: Cancer as a chronic disease. *Ann Surg* 137:683-703, 1953
- (7) GORDON-TAYLOR G: The incomputable factor in cancer prognosis. *Br Med J* 1:455-462, 1959
- (8) STEWART FW: Experiences in spontaneous regression of neoplastic disease in man. *Tex Rep Biol Med* 10:239-253, 1952



FIGURE 1.—The martyrdom of St. Agatha, by Anthony Van Dyck. (from Br J Surg 36:116, 1948).

FIGURE 2.—St. Peregrine, the patron saint of cancer and malignant diseases.

FIGURE 3.—The spontaneous cure of St. Peregrine's leg cancer.

Spontaneous Regression in Gynecologic Neoplasia¹

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SUMMARY—As I have tried to tabulate and evaluate the cases of spontaneous regression of gynecologic malignancy, several facts stand out: 1) They were infrequent. 2) The tumors most commonly undergoing spontaneous regression were the germinal epithelial tumors of the ovary; there was not any case of spontaneous regression of any other variety of ovarian neoplasm. 3) There was not one legitimate case of spontaneous regression of an invasive epidermoid carcinoma of the vulva, vagina, or uterus.—*Natl Cancer Inst Monogr* 44: 27-30, 1976.

When one embarks on an attempt to discuss the topic of spontaneous regression, the first obstacle is to define what is meant by "spontaneous" and "regression." Everson (1), Stewart (2), and Boyd (3) have generally defined spontaneous regression to include the partial or complete disappearance of a malignant tumor in the absence of any treatment or in the presence of therapy which is considered grossly inadequate by modern standards. Most authors do not make "complete" regression a necessity for inclusion. In fact, some accept a regression of tumor in one anatomic site with continual growth in others. Most do not insist on complete cure or 5-year salvage. This may seem a bit cavalier on the surface, but it is unusual for malignant neoplasia to regress when uninfluenced by acceptable techniques of therapy, and it would add a great deal to the understanding of the biology of this disease if we could gain some insight into this most infrequent and peculiar behavior.

The understanding of spontaneous regression is intimately tied to the comprehension of the immunologic aspects of host-neoplasm interaction. Even more basic is that this same interface exists at a microscopic level between emerging tumor and host resistance. An immunologic surveillance system has been envisioned as a mechanism operative in mammals to recognize and eradicate foreign antigens arising in the body. This concept of surveillance is dependent upon the existence of tumor-specific antigen. It is postulated that many microscopic tumors arise, but that few are able to break through the immunologic surveillance. Cell-mediated immunity, or so-called delayed hypersensitivity, is largely responsible for tumor immunity, and the thymus-dependent lymphocyte is the cell that seems key to this role.

Everson (1), in his series, and Stephenson et al. (4), in their accumulated series, indicate that 50% or more of the tumors acceptable as examples of spontaneous regression fall into four major categories. These are hypernephromas, neuroblastomas, malignant melanomas, and malignant trophoblastic tumors. The trophoblastic tumors, of course, are neoplasms of the product of conception, and as such, really represent homografts. Regression of this variety of

tumor might well be expected, as are rejections of organ homografts. The most surprising aspect is that it is able to progress at all. This may be related to the masking of antigenicity by the coating of tumor-specific antigen with substances such as sialomucin (5). The topic of trophoblastic tumors, however, will be adequately covered by Dr. Hertz (6), and I will turn my attention to the question of spontaneous tumor regression of female genital tumors.

In Everson's fine review article of 1967 (7), he reports a total of 176 cases that he accepts as genuine spontaneous regression. Despite the frequency of occurrence of carcinoma of the uterus in the population at large, only 4 cases of spontaneous regression of this disease were reported.

The first was that of a 33-year-old nullipara with adenocarcinoma of the uterus. Presumably this was fundal, for no actual cervical lesion was described. The tumor was extensive and the uterus was described as fixed in the pelvis. Four years later she apparently underwent a hysterectomy, and 5 years after diagnosis she had large abdominal masses and a soft tissue mass filling the vagina. Recurrence was substantiated pathologically. The patient died 7 years after diagnosis with definite regression of the abdominal masses and vaginal lesion, but autopsy did reveal "glandular carcinoma infiltrated with many calcareous pearls."

The second case was that of an elderly woman with a uterine carcinoma (cellular type not disclosed) who underwent hysterectomy. Several months later the tumor recurred in the vaginal scar. The recurrence grew rapidly, but the patient was lost to follow-up for 1 year. At that time there was no clinical evidence of tumor; the remainder of the course was unknown.

The third case was that of a 67-year-old woman with what appears to have been a mesonephroid carcinoma of the uterine body, treated initially by a radical panhysterectomy. Four months later, large abdominal and pelvic masses were noted at the time of umbilical hernia repair. Microscopic examination confirmed the diagnosis. With no further treatment, 5½ months later there was no clinical evidence of tumor. About 2 years later the patient died of other disease; there was no clinical evidence of tumor, but no autopsy was performed.

The fourth case was that of a 61-year-old female. On pelvic examination a large, Stage I (or possibly Stage II) carcinoma of the cervix was found. Pathologically this was an epidermoid carcinoma. She was treated with central radium, and 3½ weeks later the initial diagnosis of epidermoid carcinoma was again confirmed by biopsy. The patient remained clinically free of tumor and lived for 11 years without further treatment.

It is apparent that the first 3 cases of fundal adenocarcinoma probably represent partial tumor regression but do not qualify as a spontaneous cure. The fourth case, which was the only case of invasive cervical cancer, does not even represent a valid case of spontaneous regression. All of us, who have worked in the field of gynecologic oncology, realize that biopsy evidence of microscopic tumor

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3½ weeks after central irradiation does not indicate that this treatment modality failed to cure the tumor. Both this review (7) and my recent one of the literature failed to turn up a legitimate case of long-term spontaneous regression or cure of epidermoid cervical cancer.

In 1972, Baggish and Woodruff (8) reported a series of 12 cases of stromatosis. Although this lesion is usually considered to be a locally invasive neoplasm arising from endometrial stroma, 3 of the 12 patients showed evidence of distant metastases. In spite of the fact that the tumor appears microscopically benign, it clearly represents a low-grade sarcoma. One of the patients was a 50-year-old para 4-0-0-4³ who underwent an exploratory laparotomy at her local hospital for what seemed to be peritoneal carcinoma-tosis of ovarian origin. Multiple nodules were noted throughout the peritoneal cavity and one large lesion the size of a grapefruit occupied the right upper quadrant. Biopsy was performed, and this was diagnosed pathologically as a granulosa cell tumor. She was then transferred to The Johns Hopkins Hospital for radiotherapy. Review of the pathology by our gynecologic pathologist led him to believe that this might represent stromatosis. Repeat laparotomy disclosed the findings as reported above, but both ovaries were also involved and enlarged to about 7 cm in diameter. These were removed and were quite consistent microscopically with the diagnosis of stromatosis (fig. 1). Following bilateral oophorectomy, the patient did well; the easily palpable mass in the right upper quadrant gradually subsided and finally disappeared. Three years later, the patient is living and well, without evidence of pelvic or intraperitoneal disease.

There is little doubt that the endometrial stroma is under estrogen stimulation and that a neoplasm derived from this tissue might be estrogen dependent. This is also suggested by the fact that 2 of the 12 patients responded to progestogen therapy. Everson (7) notes that hormone withdrawal may account for a number of his cases of so-called "spontaneous regression."

In situ carcinoma of the cervix offers a unique opportunity to study a preinvasive carcinoma under conditions that are not available with other neoplasms. The tumor can usually be identified under colposcopic visualization, submitted to biopsy at will, and studied cytologically as frequently as desired without altering the lesion. It has a relatively slow conversion rate to invasive carcinoma, frequently as long as a decade or more. If spontaneous regression is the result of the host's immunologic system coping with a somewhat foreign system, then it would seem probable that it might be able to handle this microscopic lesion handily and with some frequency. In spite of the fact that this is a relatively common disease, and thousands of patients have been followed for variable lengths of time, it has been difficult to prove that true in situ carcinoma of the cervix ever really resolves spontaneously. The overriding problem with most of the studies in the literature is that the biopsy has been used liberally to diagnose and follow the frequently small focal lesion, and that the biopsy itself, plus the inflammatory reaction that accompanies this small surgical procedure, may account for the disappearance of the lesion. Richart and Barron (9) followed a series of 462 cases of cervical carcinoma in situ or dysplasia. In this series, including lesions less well developed than in situ carcinoma as well as in situ carcinoma, they observed only 6 cases that appeared to regress. Unfor-

tunately, the cervix is so frequently the site of acute and subacute infections that it becomes impossible to establish if very small lesions might be destroyed by the inflammatory process alone.

Ayre and Narvaez (10) have reported some 14 cases of in situ cervical carcinoma treated by a coupled tumor antigen made from pooled multiple proteins and autologous vaccine prepared by coupling protein. The authors reported some regression in all 14 patients and total regression in 5. The series is too small and the data too incomplete to form the basis for any sweeping predictions.

At the frontier of understanding spontaneous regression is the peculiar, almost symbiotic, existence between some tumors and their host. This is certainly true of in situ carcinoma of the cervix with its frequently prolonged latent period until invasive carcinoma breaks through. Jones et al. (11) made a rather extensive study of the chromosomology of in situ and invasive carcinoma of the cervix. Their data revealed that the aneuploidy associated with in situ carcinoma of the cervix was clustered in the peritriploid range and that of invasive carcinoma in the triploid range. This makes one wonder if some clonal selection might be at work. In other words, it seems at least possible that the peritriploid variety of cell remains in situ for many years, and rarely if ever invades, whereas the peritriploid clone has a greater potential for invasion. Early invasion frequently shows a more mature variety of cell responsible for the onset of stromal invasion (fig. 2).

Although there were some anecdotal accounts of spontaneous regression of ovarian carcinoma at our institution, I was unable to document this fact. Everson (7) reports 7 cases of ovarian carcinoma that he believes qualify. All 7 are germinal epithelial malignancies. In 5, regression of peritoneal implants occurred. In 2 of the 5, this took place after removal of both involved ovaries, and in another 2 of the same 5 cases, it followed removal of a unilateral cancerous ovary. All cases seemed to represent legitimate regression despite very critical evaluation of the case histories. Three patients had apparent long-term cure.

I failed to find any documented evidence of spontaneous regression of invasive vulvar carcinoma, but both Friedrich (12) and Skinner et al. (13) present what appears to be bona fide regression of "Bowenoid" atypia of the vulva. Friedrich's case was interesting and well documented: that of a 15-year-old pregnant schoolgirl, who developed a "Bowenoid" variety of in situ vulvar carcinoma as a sequel to herpetic vulvitis. The lesions consisted of numerous small hyperpigmented papular lesions. Herpesvirus, type II, antibody titer was positive, but the author was unable to isolate the active virus from fresh tissue. Electron microscopic studies demonstrated "viral particles" in the nuclei of the dysplastic cells. Postpartum, the lesions regressed spontaneously and were replaced by normal vulvar epithelium. The author believed that the cellular atypicality represented a reversible expression of the herpesvirus stimulated by the pregnant state.

Malignant tumors have appeared in a fair number of patients after organ transplantation. Presumably, this is because of the pronounced immunologic suppression that must accompany the procedure. Tumors can arise both de novo and as a tumor of the transplanted organ. Those that arise de novo are far more common. Since gynecologic tumors occur spontaneously with great frequency in the spectrum of human malignancy, it might logically be expected that in patients under heavy immunosuppression they would appear in large numbers and be prominent

³ Designation by which the first number represents number of term pregnancies; the 2d, the number of premature deliveries; the 3d, the number of abortions; and the 4th, the number of living children.

among the neoplasms that evolve. Such is not the case. Most neoplasms developing after organ transplantation have been lymphomas and epitheliomas, but very few were of the female genitalia. In the rare case of cervical cancer reported, it was difficult to ascertain if the tumor had been present prior to transplantation, and although it seems clear that immunologic competence is closely related to the appearance of human malignancy, it is perplexing not to find a full complement of gynecologic, breast, and colon tumors.

Gynecologic malignancies offer unusual opportunities for study. Uterine, cervical, and vaginal epithelial tumors continually exfoliate neoplastic cells. Colposcopy allows us to localize cellular dysplasias and in situ cancers of the cervix and vagina and to perform biopsies at will. Tissue samples of these tumors are available with little effort before, during, and after treatment. It is frequently possible to monitor tumor growth while also surveying immunologic parameters. As the field of immunology develops and more is learned about host-tumor interaction, I imagine that much information will be gleaned from the field of gynecologic oncology.

REFERENCES

- (1) EVERSON TC: Spontaneous regression of cancer. *Ann NY Acad Sci* 114:173-181, 1964
- (2) STEWART FW: Experiences in spontaneous regression of neoplastic disease in man. *Tex Rep Biol Med* 10:239, 1952
- (3) BOYD W: The spontaneous regression of cancer. *J Can Assoc Radiol* 8:45, 1957
- (4) STEPHENSON HE JR, DELMEZ JA, RENDEN DI, et al: Host immunity and spontaneous regression of cancer evaluated by computerized data reduction study. *Surg Gynecol Obstet* 133:649-655, 1971
- (5) CURRIE GA, BAGSHAWE KD: The role of sialic acid in antigenic expressions: Further studies of the Landschütz ascites tumour. *Br J Cancer* 22:843-853, 1968
- (6) HERIZ R: Spontaneous regression in choriocarcinoma and related gestational trophoblastic neoplasms. *Natl Cancer Inst Monogr* 44:59-60, 1976
- (7) EVERSON TC: Spontaneous regression of cancer. *Progr Clin Cancer* 3:79-95, 1967
- (8) BAGGISH MS, WOODRUFF JD: Uterine stromatosis: Clinicopathologic features and hormone dependency. *Obstet Gynecol* 40:487-498, 1972
- (9) RICHART RM, BARRON BA: A follow-up study of cervical dysplasia. *Am J Obstet Gynecol* 105:386-393, 1969
- (10) AYRE JE, NARVAEZ R: Carcinoma in situ: Behavior patterns and cytoimmunology. *Oncology* 27:294-304, 1973
- (11) JONES HW JR, WOODRUFF JD, DAVIS HJ, et al: The evolution of chromosomal aneuploidy in cervical atypia, carcinoma in situ and invasive carcinoma of the uterine cervix. *Johns Hopkins Med J* 127:125-135, 1970
- (12) FRIEDRICH EG JR: Reversible vulvar atypia. A case report. *Obstet Gynecol* 39:173-181, 1972
- (13) SKINNER MS, STERNBERG WH, ICHINOSE H, et al: Spontaneous regression of Bowenoid atypia of the vulva. *Obstet Gynecol* 42:40-46, 1973

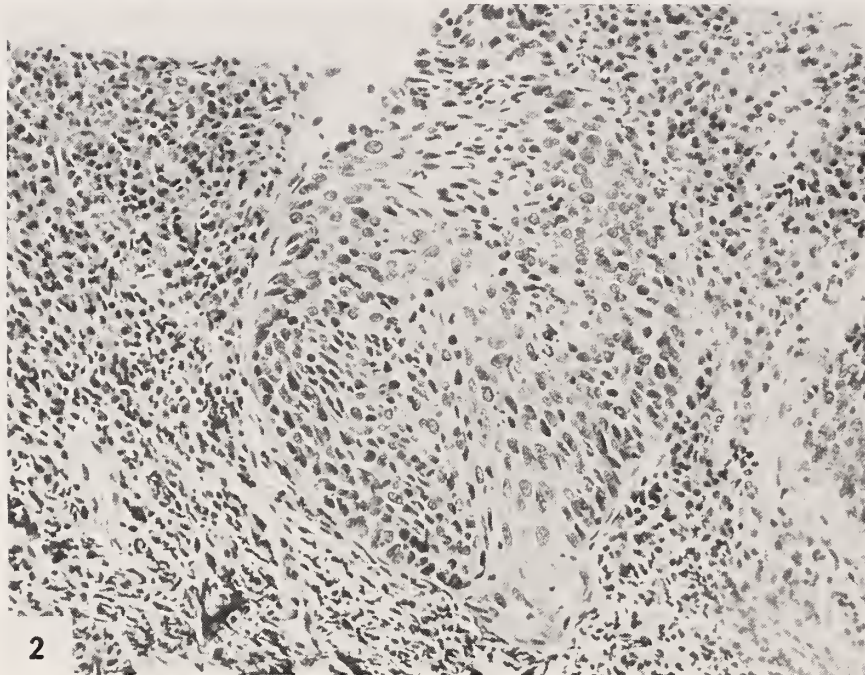
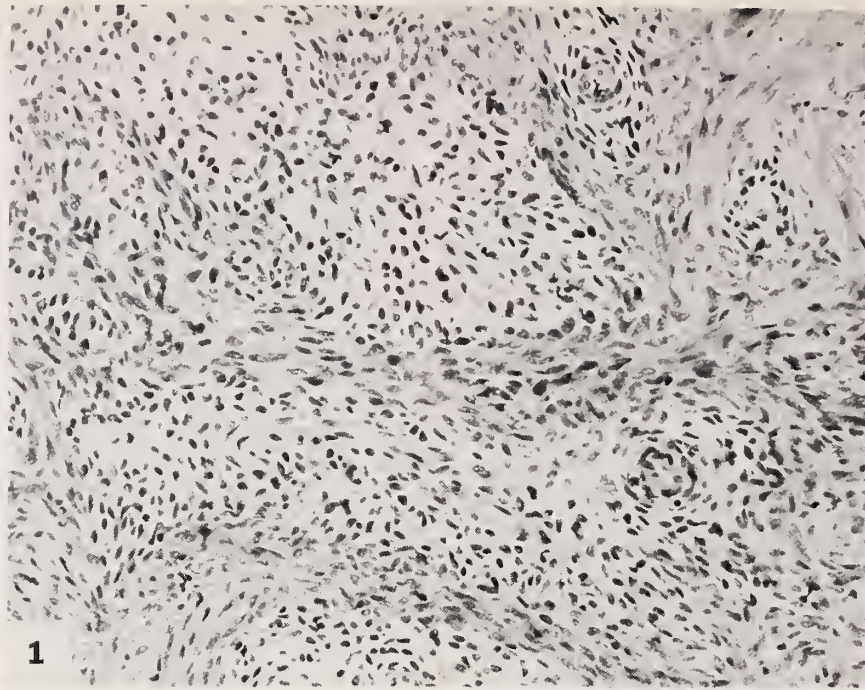


FIGURE 1.—Typical endometrial stroma infiltrating ovarian theca. Hematoxylin and eosin (H & E). $\times 240$

FIGURE 2.—Early disruption of basement membrane by relatively mature epidermoid cells. H & E. $\times 150$

Spontaneous Regression of Bronchogenic Carcinoma¹

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SUMMARY—Two cases of spontaneous regression of bronchogenic carcinoma were discovered in the literature, and the pertinent findings are described. Six equivocal cases of spontaneous regression are also presented. The reasons for the scarcity of spontaneous regression of bronchogenic carcinoma are discussed; no apparent reasons for spontaneous regression in these two cases could be determined.—*Natl Cancer Inst Monogr* 44: 31-33, 1976.

The incidence of bronchogenic carcinoma has increased by 14 times in the past 2 decades, and it was estimated that in 1974, 65,000 men and 15,000 women would die of the disease. In spite of this remarkable increase in the incidence, relatively few cases of spontaneous regression have been reported. Spontaneous regression should not be difficult to recognize because most patients are dead within 12 months of a clinical diagnosis, and it is unusual for any patient with a nonresectable tumor to survive beyond 24 months.

The medical literature printed in English between 1940 and 1973 was reviewed. For a case to be classified as true spontaneous regression, the following criteria were necessary: 1) X-ray evidence of a mass lesion, 2) unequivocal histologic diagnosis, and 3) no treatment other than biopsy. The exclusion of cases in which therapy of any kind was employed is at variance with the criteria of spontaneous regression that has been described by Everson and Cole (1), who included patients who remained well in spite of inadequate therapy. Two cases of spontaneous regression of bronchogenic carcinoma that fulfilled the criteria outlined above were found in the literature. A number of other cases of spontaneous regression of bronchogenic carcinoma have been reported. These cases are discussed, and the reasons for their exclusion from the study are described.

ANALYSIS OF CASES

Two cases of spontaneous regression of bronchogenic carcinoma were found in the literature. The patients' ages, histologic diagnoses, and subsequent clinical courses are reviewed in table 1. In both instances, careful review reveals that large, inoperable tumors were present, and total removal of the tumor by an incisional biopsy was clearly impossible.

The first case, reported by Blades and McCorkle (2), may not be a true case of spontaneous regression because of the subsequent development of apparent metastases. This patient remained well 5 years after biopsy of the original bronchogenic carcinoma, and the chest X-ray

TABLE 1.—Summary of clinical findings in 2 cases of spontaneous regression of bronchogenic carcinomas

Case No.	Age	Sex	Histology	Clinical course
1	59	M	Squamous cell carcinoma	Lesion disappeared; lesion in contralateral lung and ? skull metastases noted 5 yr later; died 6 yr later, no autopsy.
2	63	M	Squamous cell carcinoma	Lesion disappeared; patient died 12 yr later of adenocarcinoma of pancreas, no residual bronchogenic tumor found at autopsy.

revealed almost complete disappearance of the lesion in the right lung. However, 5 years after the original diagnosis had been established, there was X-ray evidence of a possible new primary lesion or metastasis in the left lung, and X-rays of the skull revealed a destructive lesion on the occipital bone. He died 6 years later, and an autopsy was not performed.

The second case, reported by Emerson et al. (3), is more convincing of true spontaneous regression. X-ray evidence showed the squamous cell carcinoma had completely disappeared, and when the patient died 12 years later of adenocarcinoma of the pancreas, there was no evidence of residual bronchogenic carcinoma at autopsy. Extensive scarring was present at the site of the original tumor, and the scar was infiltrated with plasma cells and lymphocytes.

As previously noted, a number of other cases of spontaneous regression of bronchogenic carcinoma have been reported. Bell (4) described a case of spontaneous regression in a patient with a large cell carcinoma, who survived for 10 years. No surgical therapy other than incisional biopsy was performed, but the patient received 2,100 R radiation, measured in air to a field measuring 15×18 cm, over a period of 13 days. Although it would be extremely unlikely that irradiation of this type would cure this patient, it is conceivable that this dose would eradicate some peculiarly sensitive, large-cell bronchogenic carcinomas. Euphrat (5) reported 11 cases of long-term survival in patients who received irradiation for squamous cell carcinomas, and we can add 2 others at this institution, who had oat cell carcinoma and have survived for 6 years after irradiation. In all of these cases, considerably larger doses of irradiation were used, but the dose of irradiation that is necessary to eradicate individual tumors is, of course, not known.

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Smith (6) reported 3 cases of bronchogenic carcinoma, 2 squamous cell carcinomas and 1 combination oat cell and squamous cell carcinoma; the patients survived for 7-15 years after an apparent tumor was left on the atrium following a pneumonectomy. These cases have been excluded because the presence of residual tumor on the atrium was not confirmed by histologic section. Margolis and West (7) described a 58-year-old male who underwent a right pneumonectomy for adenocarcinoma. The surgeon felt that he had not removed the entire tumor, so the case was reported as an incidence of spontaneous regression. This case was excluded because there was no histologic proof of tumor remaining in the chest following the right pneumonectomy. We can also add a case from this institution of a patient who survived for more than 5 years with a positive bronchial margin following excision of a bronchogenic carcinoma. Again however, there was no histologic proof of residual tumor remaining in the patient.

Sutton and Pratt-Johnson (8) described a bronchogenic carcinoma in a 64-year-old white female, which they reported as a case of spontaneous regression. The patient had oat cell carcinoma of the right upper lobe, which was documented by bronchoscopic biopsy. Metastases were known to be present in the humerus, in the marrow of the sternum, and in the ribs. Three months later the tumor could not be found on bronchoscopic examination of the right upper lobe, and it is conceivable that a small oat cell carcinoma was completely removed by the bronchoscopic biopsy. However, the patient had evidence of persistent metastases in the sternum and ribs in spite of the fact that the primary tumor could no longer be found on bronchoscopic examination.

Boyd (9) reported a patient with a bloody pleural effusion that contained cytologic evidence of malignant cells, but which subsided after multiple thoracenteses. The patient died 2 years later, and at autopsy, nodules of fibrous tissue were found about the hilum of the right lung. This fibrous tissue was infiltrated with an adenocarcinoma. There was no evidence of a primary lesion of the gastrointestinal tract. Although this case was reported as possible spontaneous regression or, more correctly, as a case of spontaneous remission, the report was excluded because 2-year survival with a well-differentiated adenocarcinoma is not unusual.

DISCUSSION

A review of the literature confirms the fact that spontaneous regression of bronchogenic carcinoma is rare. At least 750,000 people have died of bronchogenic carcinoma in the United States since 1950, and in this period of time only one case of unequivocal spontaneous regression has been reported. The scarcity of spontaneous regression appears to be related both to the virulence of bronchogenic carcinomas and to a lack of host resistance.

A few bronchogenic carcinomas are relatively indolent tumors that rarely metastasize. These more favorable histologic patterns include carcinoid tumors, adenocystic, mucoepidermoid, and low-grade papillary carcinomas, and certain adenocarcinomas that arise in the terminal bronchioles and grow out into the alveoli, the alveolar carcinomas. Unfortunately, the vast majority of bronchogenic carcinomas are virulent tumors, particularly when they are large enough to produce symptoms or to become detectable by a chest X-ray. Four general histologic patterns can

be recognized: the adenocarcinoma, the squamous cell, undifferentiated large-cell, and the undifferentiated small-cell carcinomas. Although most of these types of bronchogenic carcinoma grow rapidly and metastasize early in the course of the disease, at least some of them can remain as relatively indolent tumors for many years prior to a clinical diagnosis. The experience in this hospital (10) with early squamous cell carcinomas indicates that a small percentage of these lesions apparently existed *in situ* for several years; we have encountered 1 patient who has remained asymptomatic with a normal chest X-ray for the past 30 months, despite the fact that he has unequivocal cytologic evidence of squamous cell carcinoma in his sputum and the source of these cells has been localized to a specific lobar bronchus.

Garland et al. (11) and Rigler (12) have also demonstrated that X-ray evidence of both squamous cell carcinomas and adenocarcinomas can exist for many years prior to a clinical diagnosis. Whether the subsequent explosive growth phase of these tumors, which is usually associated with widespread metastases, is related to some intrinsic property in the tumor itself or to a defect in the immunologic surveillance system of the host cannot be determined. A number of investigators have demonstrated that once a clinical diagnosis of bronchogenic carcinoma is established, there is evidence of impairment of both humoral and cellular immune responses, which indicates that a lack of host resistance may account in part for the rapid death of most patients with bronchogenic carcinoma.

The reasons that spontaneous regression occurred in the 2 patients reported by Blades and McCorkle (2) and Emerson et al. (3) are not apparent. In both instances, unequivocal histologic evidence of squamous cell carcinoma existed, and there was no histologic evidence of a more favorable histologic pattern. Nonspecific stimulation of the reticuloendothelial system, such as would occur with a severe postoperative infection, has been credited by some authors for the increased survival rates observed in some patients with bronchogenic carcinoma who survived postoperative empyemas (13). One of the patients with spontaneous regression recovered from the operative procedure without complication; the other patient had a low-grade fever for several days without evidence of empyema or other significant infection. In both cases, an incisional biopsy was performed and there was no attempt at excision; this rules out the possibility of spontaneous regression occurring as a result of interference with the blood supply of the tumor. One of the 2 patients with spontaneous regression continued to smoke cigarettes after the diagnosis was established, thus eliminating the possibility, in one instance at least, of withdrawal of a known carcinogenic agent as the cause of the spontaneous regression.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966
- (2) BLADES B, MCCORKLE RG: A case of spontaneous regression of an untreated bronchogenic carcinoma. *J Thorac Surg* 27:415-419, 1954
- (3) EMERSON GL, EMERSON MS, SHERWOOD CE, et al: Spontaneous regression of bronchogenic carcinoma. *J Thorac Cardiovasc Surg* 55:225-230, 1968
- (4) BELL JW: Possible immune factors in spontaneous regression of bronchogenic carcinoma. Ten-year survival in a patient treated with minimal (1,200 R) radiation alone. *Am J Surg* 120:804-806, 1970

- (5) EUPHRAT EJ: Long arrest of epidermoid carcinoma of the lung after irradiation. *Cancer* 9:453-458, 1956
- (6) SMITH RB: Cure of lung cancer from incomplete surgical resection. *Br Med J* 2:563-565, 1971
- (7) MARGOLIS J, WEST D: Spontaneous regression of malignant disease: Report of three cases. *J Am Geriatr Soc* 15:251-253, 1967
- (8) SUTTON M, PRATT-JOHNSON JH: Spontaneous regression of carcinoma of bronchus. *Clin Radiol* 21:256-258, 1970
- (9) BOYD W: *The Spontaneous Regression of Cancer*. Springfield, Ill., Charles C Thomas, 1966
- (10) BAKER RR, MARSH BR, FROST JK, et al: The detection and treatment of early lung cancer. *Ann Surg* 719:813-818, 1974
- (11) GARLAND LH, COULSON W, WOLLIN E: The radial growth and apparent duration of untreated primary bronchial carcinoma. *Cancer* 16:694-707, 1963
- (12) RIGLER LG: Natural history of untreated lung carcinoma. *Ann NY Acad Sci* 114:755-766, 1964
- (13) TAKITA H: Effect of postoperative empyema on survival of patients with bronchogenic carcinoma. *J Thorac Cardiovasc Surg* 59:642-644, 1970

Spontaneous Regression of Hematologic Cancers¹

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SUMMARY—Spontaneous regression of hematologic cancer is extremely rare. Data gleaned from the literature and from previously unreported cases allow certain interesting general conclusions. Spontaneous remission of acute leukemia is associated with bacterial infection and is of short duration, weeks to months. Spontaneous regression of lymphoma or plasma cell dyscrasia is often of substantial duration, months or years, and frequently is associated with viral infections. Spontaneous regression of chronic lymphocytic leukemia is also of significant duration and has been associated with the occurrence of a new primary carcinoma in one-third of the cases.—*Natl Cancer Inst Monogr* 44: 35-38, 1976.

Attention was first directed to spontaneous regression of human cancer some 600 years ago when the leg tumor of a preterminal middle-aged man allegedly mysteriously resolved overnight, restoring the patient to perfect health. In recognition of the important role played by the *host* in such a noteworthy host-tumor interaction, the patient was later canonized Saint Peregrine. Boyd (1) suggested that spontaneously regressing tumors in man might be referred to as Saint Peregrine tumors.

Data collected over the next 600 years concerning the spontaneous regression of cancer have for the most part continued to be anecdotal, and postulated mechanisms have often been as mysterious as those implied by the canonization of Saint Peregrine.

Case histories of apparently bona fide examples of regression of human solid tumors without specific therapy have been collected and analyzed in at least two monographs, one by Boyd (1) and one by Everson and Cole (2). Hematologic cancers have not been scrutinized so carefully in modern times. The purposes of this paper are to tabulate and analyze previously reported examples of spontaneous regression of hematologic cancer, to describe a number of previously unreported, well-documented examples of this phenomenon, and to speculate in broad terms their significance.

SPONTANEOUS REMISSION OF ACUTE LEUKEMIA

The syndrome of ineffective granulocytopoiesis masquerading as acute myeloblastic leukemia in Down's syndrome has often been cited as support for the concept of spontaneously regressing acute leukemia (3). There are at least 25 published examples of this syndrome, which occurs predominantly in newborns. In all reported cases, the findings suggesting leukemia (leukocytosis, thrombocytopenia, organomegaly, and skin infiltration) resolved

without specific therapy over a period of weeks or months. This syndrome is probably best referred to as "transient leukemoid reaction," since similar findings are seen with a myriad of neonatal problems, including ABO incompatibility (4).

A convincing example of transient spontaneous remission in a case of untreated congenital acute myeloid leukemia was reported by Van Eys and Flexner (5). The patient was not a mongoloid and had a complete clinical and hematologic remission lasting 3½ months. The patient had a subsequent relapse and died after an unsuccessful chemotherapeutic trial. There were no associated phenomena, such as infection, that might suggest a mechanism for this patient's remission.

Southam et al. (6) collected from the literature 57 cases of spontaneous regression of acute leukemia in children and adults and summarized them in 1951. In 12 of the cases, the remission was complete, as proven by bone marrow biopsy. The median duration of remission was 17+ weeks, and in many patients the onset of remission was preceded by presumed or documented bacterial infection.

In his report, Southam added 13 cases from Memorial Hospital in New York (table 1). Seven of the patients had significant but only partial remission; 2 had complete remission documented by bone marrow biopsy; and 4 had presumed complete remission without bone marrow documentation. Eleven of the 13 patients had significant febrile reactions prior to the onset of spontaneous remission, and 6 had proved bacterial infection.

Bassen and Kohn (7) and Bierman et al. (8) subsequently reported 7 children with ALL who improved after bacterial or viral infection or fever of undetermined origin (FUO). Four of these children apparently had complete remissions lasting 1-4 months, and 1 patient had multiple spontaneous remissions (table 2).

In 1951, Diamond and Luhby (9) reviewed experience with spontaneous remission of childhood leukemia. They found an incidence of complete spontaneous remission of 4.5% in 300 children with acute leukemia and a mean duration of remission of 10 weeks. The children who experienced these remissions had several things in common. They all had ALL with normal or low white blood cell counts, and complete remission was preceded by marked leukopenia and marrow hypoplasia associated with severe pyogenic infection.

Several years earlier, Dreyfus (10) had reached almost identical conclusions after reviewing the literature and examining his experience in France. On the basis of his review of spontaneous regression of acute leukemia, he suggested that acute idiopathic aplastic anemia in children might represent a phase of acute leukemia. Some years later, Perner et al. (11) made an exhaustive review of the literature concerning spontaneous regression of acute leukemia and arrived at conclusions essentially similar to those of Diamond and Luhby and Dreyfus.

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TABLE 1.—*Spontaneous remission in childhood acute leukemia (6)*

Group ^a	Num-ber of pa-tients	Cell type ^b	Remis-sion dura-tion (wk)	Comment
CR	2	ALL	12-16	Both had fever; 1 had proved infection.
Presumed CR	4	ALL	3-9	Two had fever; none had proved infection.
PR	3	AML	2-12	All had fever; 5 had proved infection.
	4	ALL		

^a CR=complete remission proved by bone marrow examination; presumed CR=complete remission not documented by bone marrow examination; PR=substantial improvement but not complete (partial) remission.

^b ALL=acute lymphocytic leukemia; AML=acute myelocytic leukemia.

TABLE 2.—*Spontaneous remission in childhood ALL associated with infection*

Patient No.	Age	Infection	Type ^a	Remission duration (mo)	Ref-erence
1	15 mo	FUO	CR	2, 1, 5, 4, 3	(7)
2	14 yr	<i>Staphylococcus aureus</i>	CR	1, 5	(8)
3	2 yr	<i>S. aureus</i>	CR	1, 5	(8)
4	2 yr	<i>S. aureus</i>	Imp	0, 5	(8)
5	9 yr	<i>S. aureus</i>	Imp	1, 25	(8)
6	4 yr	Varicella	CR	1, 0	(8)
7	6 yr	Varicella + <i>S. aureus</i>	PR	1, 25	(8)

^a Imp=improvement.

Members of the American Society of Clinical Oncology were canvassed for information on previously unreported patients with hematologic cancer who experienced spontaneous regression. I am indebted to Drs. Bayard Clarkson, Donald Hayes, Chris Zarofonotis, and William Regelson for the information (table 3) on patients with acute leukemia whose disease remitted without specific therapy. These 10 cases of spontaneously regressing acute leukemia have some noteworthy characteristics in common. All patients had brief remission of their disease which lasted 1-5 months and was associated with severe bacterial infection, and they all had subsequent relapses and died of leukemia. Only half the patients had ALL; only 2 of the 10 were older than 25 years.

Data such as these may have influenced Sadoff (12) to postulate a mechanism of action for cancer chemotherapeutic agents based on inhibition of immunologic function. Sadoff suggested that leukopenia per se might be the most significant action of such drugs, rather than direct tumor cell kill as most data would suggest. Sadoff postulates that leukopenia may reflect B-cell suppression, which in turn might be associated with decreased blocking-antibody production. Such a situation may render tumor cells more susceptible to host defense mechanisms.

SPONTANEOUS REGRESSION OF LYMPHOMA AND PLASMA CELL DYSCRASIAS

The most impressive report of regression of lymphoma without specific therapy is that of Bluming and Ziegler

(13). Their patient was an 8-year-old African with Burkitt's lymphoma of the orbit. Complete remission occurred directly after the clinical onset of a measles infection, and the patient remained disease free for 4+ months (table 4). A more complete description of this case is presented by Ziegler (14).

A probable case of complete remission of lymphosarcoma in association with probably viral hepatitis was reported by Weintraub (15). That patient died of congestive heart failure 22 months after the onset of remission of his lymphoma, and there was no clinical evidence of lymphoma at the time of his death. No autopsy was performed, however.

Three well-documented spontaneous regressions of plasma cell dyscrasias were gleaned from the literature. London (16) reported a complete spontaneous remission of multiple

TABLE 3.—*Previously unreported examples of complete spontaneous remission of acute leukemia^a*

Pa-tient No.	Age (yr)/sex	Cell type ^b	Remission-associated events	Remission duration (mo)
1	22/F	ALL	<i>Escherichia coli</i> sepsis	1-2
2	12/M	ALL	<i>Staphylococcus furunculosis</i>	1-2
3	10/F	ALL	<i>E. coli</i> + aerobacter peritonitis	1, 25
4	5/M	ALL	<i>E. coli</i> peritonitis	3
5	12/M	ALL	<i>E. coli</i> sepsis	4, 5
6	11/M	AML	Otitis media	2
7	40/M	AML	Pneumonia, empyema	4-5
8	25/F	AML	α -Streptococcus + aerobacter periton-sillar abscess	2
9	75/F	AMOL	Pneumonia	3
10	17/F	AMML	<i>E. coli</i> peritonitis	1, 5

^a All patients subsequently died of leukemia.

^b AMOL=acute monocytic leukemia; AMML=acute myelomonocytic leukemia.

TABLE 4.—*Complete spontaneous regression of lymphoma and plasma cell dyscrasia*

Pa-tient No.	Ref-erence	Age (yr)/race/sex ^a	Remis-sion du-ration (mo)	Cell type	Associated infections ^b
Lymphoma					
1	(14)	8/N/M	4+	Burkitt's	Measles
2	(15)	68/C/M	22	Lymphocytic	Hepatitis
Plasma cell dyscrasia					
1	(16)	39/C/F	24	Myeloma	Hepatitis, Herpes zoster
2	(17)	57/N/M	6+	Waldenström's	None
3	(18)	59/C/M	60	Waldenström's	Au + hepatitis

^a N=Negro; C=Caucasian.

^b (15) and (16) were written before the test for Australian (Au) antigen was available.

myeloma associated with probable viral hepatitis and herpes zoster. The remission lasted 2 years, after which time the patient had a relapse and died of progressive myeloma. Nutter and Kramer (17) described a patient with Waldenström's macroglobulinemia, who normalized all of his clinical and laboratory signs with no therapy and no known associated infection. That patient remained in remission for at least 6 months. Wolf et al. (18) also reported a patient with spontaneously regressing Waldenström's macroglobulinemia. The remission onset coincided with the development of Au+ hepatitis and persisted for 5 years until the patient died of pneumococcal sepsis. Although there was no laboratory or clinical evidence of plasma cell dyscrasia at the time of death, the occurrence of pneumococcal sepsis suggests that some derangement of immunologic function may have occurred terminally in this patient, despite the absence of other evidence of plasma cell dyscrasia.

Data on 3 previously unreported patients who experienced spontaneous regression of lymphoma have been described to me by Drs. Lowell Irwin, V. K. Vaitkevicius, and Sherwood P. Miller (table 5). These patients had complete regression of lymphoma not associated with specific therapy, infection, or other noteworthy circumstances. The remissions were of long duration and appear to have been truly spontaneous.

In contrast with remission of acute leukemia, spontaneous regression of the lymphomas or plasma cell dyscrasias appears to be of long duration, either associated with *viral* infection or no known infection.

Because of the high incidence of infection associated with spontaneous remission in the hematologic malignancies, a number of earlier workers attempted to treat these disorders with various viral and bacterial preparations (19-21). Occasional beneficial effects were seen, but most methods were too toxic and fell by the wayside with the advent of chemotherapy. However, Miller and Nicholson (22) recently reported some striking results when Coley toxins were given as adjuvants to surgery and/or radiation therapy to patients with primary reticulum cell sarcoma of the bone. They claim that such therapy improves by more than 50% the 5-year survival rate of patients with this relatively uncommon type of lymphoma. Their work deserves confirmation.

The circumstances surrounding the spontaneous regression of chronic lymphocytic leukemia appear to be strikingly different from those associated with cryptogenic regression of other hematologic cancers (table 6). Remissions are prolonged and not associated with infection. However, 4 of 12 patients developed a carcinoma at the time of onset of the leukemia remission or several years

TABLE 6.—*Spontaneous remission in chronic lymphocytic leukemia*

Pa-tient No.	Ref-er-ence	Age(yr)/race/sex	Type ^a	Remis-sion dura-tion (yr)	Comment
1	(23)	48/C/M	CR	11+	
2	(24)	—	CR	9	Died of colon cancer
3	(25)	52/C/M	CR	8+	
4	(25)	54/C/M	CR	8+	
5	(25)	47/C/M	CR	7+	
6	(25)	55/C/M	CR	1.5	Died of bronchogenic carcinoma
7	(25)	45/C/M	GPR	4+	Developed squamous cell carcinoma of ear
8	(26)	69/?/F	GPR	5+	
9	(26)	64/?/M	CR	1.5	Died of bronchogenic carcinoma
10	(26)	37/C/F	GPR	1+	Died of lymphoma
11	(26)	47/?/M	GPR	1	Relapsed
12	(27)	52/C/F	CR	5+	

^a GPR=good partial remission.

later. In fact, the high incidence of carcinoma suggests that such a tumor should be diligently looked for in any chronic lymphocytic leukemia patient whose leukemia spontaneously and progressively improves.

It is tempting to speculate that if some immunologic mechanism such as antigenic competition (28) is responsible for the improvement in the leukemia of these patients, therapy with tumor-specific antigenic material from carcinomas might be effective in chronic lymphocytic leukemia. It is, of course, currently in vogue to speculate in this way. That such speculation might prove profitable is suggested by Webb and Smith (29), who have recently postulated that viral infection in cancer patients might be manipulated to therapeutic advantage, and by Ivanovsky (30), who has demonstrated interaction between hepatitis and animal leukemia viruses. Brown et al. (31) have more recently suggested, however, that infection with hepatitis virus might facilitate the development of lymphoma in the compromised host, such as transplant recipients.

It is hoped that studies of spontaneously regressing mouse leukemia, such as those of Rich and Clymer (32), and studies on the mechanism of regression of chicken lymphoma following viral infection, such as those of Love and Sharpless (33), will provide data that ultimately will be beneficial to the patient with a hematologic cancer.

We and others have become aware of the fact that a large number of patients with malignant lymphoma give a history of waxing and waning lymphadenopathy prior to diagnosis. Such patients have often had multiple node biopsies that yielded inconclusive or negative results prior to the establishment of the true diagnosis. These patients upon careful study may yield clues concerning the mechanism of spontaneous regression of lymphoma in man. In addition, long-term, apparently disease-free survivors of Hodgkin's disease have often been found at autopsy to have clear histologic evidence of occult lymphoma. Such

TABLE 5.—*Previously unreported examples of complete remission of lymphoma*

Pa-tient No.	Age (yr)	Cell type	Remission-associated events	Remis-sion dura-tion (yr)	Subsequent course
1	22	Lymphocytic	Peritonitis	29	No evidence of disease
2	53	Hodgkin's	None	10	No evidence of disease
3	38	Histiocytic	None	13	Died of lymphoma

patients have been reported by Strum and Rappaport (34) and by Thomas and Berard (35). They may also provide insight into the mechanism of spontaneous regression of lymphoma.

Finally, I would like to suggest that attention also be paid to other than immunologic conditions surrounding spontaneous regression of cancer. Heat alone, for instance, has been shown by Crile (36) to cause regression of a number of animal tumors including a dog lymphoma, and Halikowski et al. (37) have claimed that a purine-free diet can significantly prolong remission duration in acute leukemia.

REFERENCES

- (1) BOYD W: The Spontaneous Regression of Cancer. Springfield, Ill., Charles C Thomas, 1966
- (2) EVERSON T, COLE W: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966
- (3) NAGAO T, LAMPKIN BC, HUG G: A neonate with Down's syndrome and transient abnormal myelopoiesis: Serial blood and bone marrow studies. *Blood* 36:443-447, 1970
- (4) ROSNER F, LEE SL: Down's syndrome and acute leukemia: Myeloblastic or lymphoblastic? *Am J Med* 53:203-218, 1972
- (5) VAN EYS J, FLEXNER JM: Transient spontaneous remission in a case of untreated congenital leukemia. *Am J Dis Child* 118:507, 1969
- (6) SOUTHAM CM, CRAVER LF, DARGEONE HW, et al: A study of the natural history of acute leukemia with special reference to the duration of the disease and the occurrence of remissions. *Cancer* 4:39-59, 1951
- (7) BASSEN FA, KOHN JL: Multiple spontaneous remissions in a child with acute leukemia. *Blood* 7:37-46, 1952
- (8) BIERMAN HR, CRILE DM, DOD KS, et al: Remission in leukemia of childhood following acute infectious disease. *Cancer* 6:591-605, 1953
- (9) DIAMOND LK, LUHBY AL: Pattern of "spontaneous" remissions in leukemia of childhood observed in 26 of 300 cases. *Am J Med* 10:238-239, 1951
- (10) DREYFUS B: Les rémissions de la leucémie aigue. *Sangre* 1:35-40, 1948
- (11) PELNER L, FOWLER GA, NAUTS HC: Effects of concurrent infections and their toxins on the course of leukemia. *Acta Med Scand [suppl 338]*: 1-47, 1958
- (12) SADOFF L: Mechanisms of action of cancer chemotherapy drugs: An alternative hypothesis based on inhibition of immunologic function. *Cancer Chemother Rep* 56:559-562, 1972
- (13) BLUMING AZ, ZIEGLER JL: Regression of Burkitt's lymphoma in association with measles infection. *Lancet* 2:105-106, 1971
- (14) ZIEGLER JL: Spontaneous remission in Burkitt's lymphoma. *Natl Cancer Inst Monogr* 44:61-65, 1976
- (15) WEINTRAUB LR: Lymphosarcoma remission associated with viral hepatitis. *JAMA* 210:1590-1591, 1969
- (16) LONDON RE: Multiple myeloma: Report of a case showing unusual remission lasting two years following severe hepatitis. *Ann Intern Med* 43:191-201, 1955
- (17) NUTTER DO, KRAMER NC: Macroglobulinemia. Report of a case with unusual spontaneous recovery. *Am J Med* 38:462-469, 1965
- (18) WOLF RE, RIEDEL LO, LEVIN WC, et al: Remission of macroglobulinemia coincident with hepatitis. *Arch Intern Med* 130:392-395, 1972
- (19) TIGERTT WD, CROSBY WH, BERGE TO, et al: The virus of Venezuelan equine encephalomyelitis as an antineoplastic agent in man. *Cancer* 15:628-632, 1962
- (20) WHEELOCK EF, DINGLE JH: Observations on the repeated administration of viruses to a patient with acute leukemia. *N Engl J Med* 271:645-651, 1964
- (21) NEWMAN W, SOUTHAM CM: Virus treatment in advanced cancer. *Cancer* 7:106-118, 1954
- (22) MILLER TR, NICHOLSON JT: End results in reticulum cell sarcoma of bone treated by bacterial toxin therapy alone or combined with surgery and/or radiotherapy (47 cases) or with concurrent infection (5 cases). *Cancer* 27:524-548, 1971
- (23) CHERVENICK PA, BOGGS DR, WINTROBE MM: Spontaneous remission in chronic lymphocytic leukemia. *Ann Intern Med* 67:1239-1242, 1967
- (24) BOUSSER J, ZITTOUN R: Rémission spontanée prolongée d'une leucémie lymphoïde chronique. *Nouv Rev Fr Hematol* 5:498-501, 1965
- (25) DURANT JR, FINKBEINER JA: "Spontaneous" remission in chronic lymphatic leukemia? *Cancer* 17:105-113, 1964
- (26) HAN T, SOKAL JE: Spontaneous remission of leukemic lymphoproliferative disease. *Cancer* 27:586-595, 1971
- (27) REICH C: Apparent recovery in chronic lymphocytic leukemia. *JAMA* 170:169-171, 1959
- (28) PROSS H, EIDINGER D: In vitro studies of "antigenic competition." II. Reconstruction of the immune defect and the relationship between antigen-induced suppression and non-specific enhancement. *Immunology* 25:269-281, 1973
- (29) WEBB HE, SMITH CE: Viruses in the treatment of cancer. *Lancet* 1:1206-1208, 1970
- (30) IVANOVSKY DI: Interaction between hepatitis and animal leukosis viruses. *Acta Virol (Praha)* 16:91, 1972
- (31) BROWN RS, SCHIFF M, MITCHELL MS: Reticulum cell sarcoma of host origin arising in a transplanted kidney. *Ann Intern Med* 80:459-463, 1974
- (32) RICH MA, CLYMER R: Host response in spontaneous regression of murine leukemia. *Cancer Res* 31:803-807, 1971
- (33) LOVE R, SHARPLESS GR: Studies on a transplantable chicken tumor (RPL-12 lymphoma). II. Mechanism of regression following infection with an oncolytic virus. *Cancer Res* 14:640-647, 1954
- (34) STRUM SB, RAPPAPORT H: The persistence of Hodgkin's disease in long-term survivors. *Am J Med* 51:222-240, 1971
- (35) THOMAS LB, BERARD CW: Hodgkin's disease: Relationship of histopathological type at diagnosis to clinical parameters and to histological progression and anatomical distribution at autopsy. *Gann Monogr* 15:253, 1973
- (36) CRILE G JR: Selective destruction of cancers after exposure to heat. *Ann Surg* 156:404-407, 1962
- (37) HALIKOWSKI B, ARMATA J, GARWICZ S: Low-protein purine-free diet in treatment of acute leukemia in children: Preliminary communication. *Br Med J* 1:519-521, 1966

DISCUSSION

B. Castleman: Do any of the speakers want to quiz other speakers or would any of the audience like to ask any questions?

Question from audience: Dr. Schirmer, the question is, do you ordinarily practice removal of the kidney when the patient has a known metastasis in the lung, in the hope that there will be spontaneous regression?

H. Schirmer: The answer is no. Spontaneous regression of metastatic hypernephroma is so rare that it should not even be considered when deciding whether or not to do a nephrectomy.

Castleman: In that connection, Dr. Schirmer, assuming you did remove a primary where there was no lung metastasis and then a lung metastasis appeared, do you consider the disease-free interval length to help you decide whether or not to remove the metastasis surgically?

Schirmer: That is a very difficult question to answer, Dr. Castleman. Generally, we do not recommend removal of solitary pulmonary metastases, because too often multiple lesions are found at surgery or soon after. There are others who have a more aggressive approach, however.

P. Wiernik: I think what Dr. Schirmer says is a bit controversial, and I rise just to make a comment about it. I do think that metastatic hypernephroma should be excised from the lung, particularly a solitary lesion and particularly if it showed up 2 years after the original tumor was removed. The current index that others use is doubling time of the tumor, and it has been demonstrated that if this time is greater than 40 days, benefit from excision of these tumors may be expected. If the tumor doubling time is less than 30 days, no benefit to the patient can be expected.

Comment from the floor: I rise to agree with this last speaker. In my experience, excision of single, isolated pulmonary metastases of hypernephroma has on occasion resulted in prolonged disease-free survival.

A. Bluming: Dr. Serpick gave us rather distressing figures on the survival of patients with colonic carcinoma. I wonder if either Dr. Serpick or one of the pathologists would speculate on the markedly contrasting survival figures for metastatic carcinoid syndrome, for which survivals of many years, up to 25 years, have been reported. Certainly the origin of the two—colonic carcinoma and carcinoid—are not terribly dissimilar.

A. Serpick: Actually, colonic carcinoma and carcinoid are very dissimilar. Carcinoids of the appendix are almost never malignant. Carcinoids of the small bowel have a very low malignant potential and yet can metastasize to the liver and produce the carcinoid syndrome without progressively interfering with liver function or, in fact, survival. Carcinoid tumors of the colon are very much more malignant. How malignancy and anatomic location of a given tumor type interact is entirely unclear. Adenocarcinoma of the intestine does not show such variation in malignancy with anatomic location.

Castleman: Well, I can cite a very fascinating case (which I personally saw and had something to do with) of an individual who was operated on (I think it was in 1915) at Massachusetts General Hospital by Dr. Daniel Fisk

Jones, who is a famous abdominal surgeon, for a small-bowel obstruction. When the surgeon opened the abdomen, he found a tumor in the small bowel, with a huge mass of mesenteric nodes adjacent to it. He obviously could not remove it so he did a bypass operation. I think it was some 20 years later when I did a postmortem examination on this same man, who had died following a prostate operation. He was in his 70's or 80's at that time, and there sitting in the small bowel was the same tumor, the same mass of mesenteric lymph nodes, exactly the way they were previously observed. So the carcinoid tumor is a queer beast. Sometimes it is not even a beast. Other questions?

C. Southam: My communication will be very brief and straightforward. Several years ago at Memorial and Sloan-Kettering, I carried out extensive studies of the autotransplantation of human cancer in patients with incurable cancer, mostly gynecologic cancers. These studies were to detect evidence of resistance to cancer and to study these mechanisms. The studies I would like to mention were made possible by assistance and cooperation of several medical and surgical colleagues and especially by the long collaboration of Dr. Alexander Brunschwig and Dr. Arthur Levin. The immediate results of those studies were interpreted as revealing cell-mediated immunologic resistance to the implanted cancers and were reported in many papers and at various meetings. However, in those written and oral reports our emphasis was on the resistance to the initiation of tumor growth. Only once did we report that some regressions of the autochthonous tumor nodules also occurred. That report was nearly 8 years ago and appeared as an abstract at the International Cancer Congress in Tokyo. We had 141 patients in whom one or more subcutaneous nodules developed at some site of the autotransplanted tumor cells. Of greatest importance is that we saw complete, spontaneous regression in 17 patients, which constitutes 12% of the total of 141. This complete spontaneous regression of the nodule occurred after the nodule had been present for a period up to 6 weeks after the transplantation. Three of those patients had cervical cancer; 2 had ovarian cancer. Two of the patients had had no treatment whatever during the period that the nodule grew and subsequently regressed. The other 3 had pelvic irradiation, but these implants were in the thigh and the scatter into the thigh would be well below 1% of the irradiation that was directed into the tumor mass in the pelvis. So I think we can say that this represents a spontaneous type of regression. Perhaps you might like to refer to it as an abscopal effect of irradiation in those patients, but that, of course, is popularly thought to be an immunologic mechanism. In addition, there were 12 patients who showed complete spontaneous regression unrelated to any direct therapy, but in those 12, the nodules were not demonstrated to be present for longer than 6 weeks.

Castleman: Well, I do not want to leave without proving I really am a skeptic. I would like to discuss the first case that Dr. Baker accepted unequivocally. In that patient, the lung lesion and the skull lesion, without an autopsy to show otherwise, obviously could be inflammatory or something else; I think that case is not quite clear-cut.

Emerson's case is very interesting, and I would like to ask where the lesion was? I do that for this reason: It has been well documented that pulmonary embolism can cause squamous metaplasia of the bronchus and invasion of the area by squamous tissue, squamous cells that could definitely simulate squamous carcinoma. The same thing happens in the prostate, and it has been pointed out that this could be mistaken for carcinoma. Now it is interesting that Emerson's patient had a carcinoma of the pancreas, which we know is very apt to produce peripheral thrombosis, and the possibility of embolus as the first sign with squamous metaplasia is real. That kind of thinking would certainly tie this case together. So I would like to ask just where that tumor was and is this documented?

R. R. Baker: It is difficult to defend somebody else's report, which is the disadvantage of reviewing the literature, I suppose. I think, however, that it would be terribly unusual for somebody to develop thrombophlebitis from a carcinoma of the pancreas and then die of it 6 years later.

Linder: I am not an M.D., so I am here to ask you for advice in thinking about cancer among other things. One of the speakers brought out a small point that reminded me of some things that had been said at the 1974 meeting in Atlantic City of the Federation of American Societies for Experimental Biology. Namely, he

was speaking about carcinoma in situ developing into invasive carcinoma and pointing out that the cells that appear to be invading seem to be more differentiated than some of the ones that were in the carcinoma in situ itself. What was brought out in Atlantic City was that there have been some animal experiments in which single malignant cells transplanted to animals caused the development of a tumor that always included undifferentiated cells as well as differentiated cells. In the transplantable tumors that I use, there is always a large proportion of relatively differentiated cells within the tumor, along with the more malignant, less differentiated cells. Perhaps the differentiated cells have developed from that undifferentiated malignant cell which was transplanted. What do pathologists think about this question?

Castelman: I think it was Dr. Julian who talked about that.

C. Julian: I think the observation is valid. Too many people think that it is not. In early invasion of cervical carcinoma, differentiated cells are seen. I think that these cells derive from more poorly differentiated cells.

H. W. Jones, Jr.: The only thing I can contribute is the fact that when one studies the chromosomes of the noninvasive and the invasive parts of nodules in carcinoma of the cervix, they are identical, even though histopathologically, the cells may demonstrate variable differentiation.

INTRODUCTION: AFTERNOON SESSION

I cannot contribute in a substantive way to the discussion of spontaneous regression; my brief professional career has occurred in an era of therapeutic activism, and so I am not sure I have ever had a chance to observe the phenomenon that we are discussing. It is interesting to hear the reports of those who have had that personal experience and studied the matter. It is interesting to see skepticism expressed, and I am sure a healthy degree of skepticism is necessary if we are going to think about the matter critically and if we are going to communicate with one another effectively. Sometimes I think we tend to reject, under one guise or another, phenomena that we cannot readily explain, and I hope we will not push aside a happening of nature that might help us to a greater understanding of disease processes.

There is another thing that perhaps we tend to do from time to time and that is to explain things we see in terms and words with which we are most familiar. As I hear and perhaps anticipate a bit of what is to come, I am reminded, at least from my own thinking, that there are a great many possible explanations. I think that the people who follow on the program have a great responsibility. I hope they will attempt to present some hypotheses that will result in practical experimentation which leads to useful new knowledge.

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Spontaneous Regression of Cancer Evaluated by Computerized Data¹

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SUMMARY—Where immunologic integrity wanes, clinicians observe a greatly increased incidence of neoplasia. Factors in this phenomenon are reviewed. With complete and detailed recorded data on patients who have exhibited spontaneous regression of cancer, a systematic data-reduction approach becomes a practical reality, without the inherent handicaps of missing details in patient histories or anecdotal and often conjectural observations. Presented here is the plea for detailed recording of complete histories of all carcinoma patients who exhibit the phenomenon of spontaneous regression. Physicians of several decades ago generally considered as unrelated much data deemed important to us today.—*Natl Cancer Inst Monogr* 44: 43-47, 1976.

This startling phenomenon of a malignant growth abruptly ceasing, regressing, and even disappearing was first brought to my attention by Dr. Evarts A. Graham in 1948, while I, as an intern, was assisting him during surgery at Barnes Hospital in St. Louis, Missouri. Several times later, we discussed how these intriguing events might best be studied. What common features might these patients present? How do they differ from less fortunate cancer patients? Later, at Bellevue Hospital in New York, I was fascinated by the accounts provided by Dr. Bradley Coley and Dr. Herbert Willy Meyer, who recalled the observations of their famous fathers near the turn of the century regarding the occasional regression of a cancer on which an erysipelas infection had fortuitously been superimposed.

Through the years a brief report would occasionally appear to whet one's interest and again challenge one's imagination. In 1966, the focus on spontaneous regression of cancer was greatly accentuated by the publication of Everson and Cole's superb monograph (1) on the subject. These two highly regarded and thoroughly reputable surgeons brought an increased aura of respectability to this sometimes ridiculed observation of presumed spontaneous regression. Today, it is a real privilege to be invited to participate in this first symposium on spontaneous regression of cancer. Most significantly, I believe, is that 24 of the 32 listed faculty come from a wide variety of disciplines other than surgery. Spontaneous regression is being viewed from the perspective of the immunologist, pathologist, pediatrician, radiologist, pharmacologist, dermatologist, internist, gynecologist, and even the neuropsychiatrist. New observations, new techniques, and new considerations prompt us to take still another look at this fascinating group of patients in the hope that the explanation may become more evident.

About 7 years ago, applying Sutton's law³ to this problem, we turned to our colleagues in computer science for

assistance. Would it be possible to extract worthwhile information regarding spontaneous regression cases, which had been coded and evaluated, by computerized data-reduction methods? Could the computer be used as a research tool to help discover new information? Dr. Donald Lindberg, a bona fide pioneer in the application of computers to medical problems, encouraged us to try.

It seemed reasonable that our ultimate goal be the establishment of a minimal subset of variables to characterize the population with spontaneous regression and to distinguish it from cancer patients not experiencing this phenomenon. We hope to construct this control matrix as soon as possible and to improve our own capability of handling any new data that may become available. Although the problem of missing observations is an extremely difficult one, we would make a plea that new cases include as much data as possible. In addition, we hope soon to develop the capability of sending out trained physicians to seek personally whatever additional data is available, to interview patients whenever possible, and eventually to provide our program with the capability of predicting which cancer patients are at least potentially in the reach of spontaneous regression, recognizing, of course, that we may not be dealing with a homogenous group and that subpopulations or clusters may appear.

As Dr. Lindberg cautions, most of us are imbued with the belief that most syndromes are triads, and we need to adapt ourselves to the realization that many more than three variables may be involved.

Any future analysis of cases of spontaneous regression ideally should include more adequate follow-up studies. In our own group of 224 patients, for which we relied heavily on Dr. Cole's (1) group, only 14 patients are known to have survived more than 5 years after evidence of spontaneous regression, although 140 were alive at the time of the original report. Thirty-two patients are known to have succumbed to their malignancy. One of the best documented cases of spontaneous regression, and certainly well known to most of us interested in this subject, is that reported by Sumner (2). Dr. Sumner reported to me recently that this woman, who had widespread cutaneous malignant melanoma almost 25 years ago, is now 54 years old and in excellent health. The histology was reviewed and reported (3) to the Ewing Society several years ago. Today the areas in her scalp and around her body continue to show depigmentation at the sites of regression. A man who subsequently was given transfusions with 250 ml of her blood lived 7 years and died of an unrelated disease; at autopsy this patient was free of any evidence of melanoma despite widespread osseous and subcutaneous involvement at the time of the transfusion.

We are all aware of the flurry of intense activity toward investigation of a potential bolstering of the host defense mechanisms and, in general terms, toward a stimulation of the body's thymic-lymphoreticular-endothelial system. Freund's adjuvants, vaccinia virus, dinitrochlorobenzene, BCG, and other agents are being used in this behalf. Our first case is shown in figure 1. This patient was a professor at the University of Missouri who had primary melanoma of the leg, followed in 6 weeks by an inguinal node dis-

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³ Sutton's Law: "When you order a laboratory test or diagnostic procedure, choose the one that will yield the desired information—because that's where the money is" was proposed by Dr. William Dock in reference to Willie Sutton's famed statement that he robbed banks "because that's where the money is" (*Medical Tribune* 15:2, April 24, 1974).

section. He came in with stasis melanosis or satellitosis up and down the leg. He had a perfusion without apparent benefit, and we subsequently injected BCG into these metastatic lesions. The progression of events toward an eventual vitiligo was very exciting.

By coding all patients associated with what we thought were inadvertent reticuloendothelial stimulants of some kind, we were able to gather at least 77 instances; in addition, 62 patients experienced an infection or persistent temperature elevation prior to regression of their disease. Significant changes in the hormonal milieu of the patient were recorded in 45 instances. Thus one or more of these three factors were present in 184 of 224 patients. Present events tend to give even more credibility to this nagging suspicion that accidental infections, bacterial toxins, and other agents acting as adjuvants affecting the lymphoreticuloendothelial system enhance the immune mechanism and host resistance. Several impressive reports document the observation, for example, of unusually long-term survivors esophageal and bronchogenic carcinoma complicated by of an infection.

Over a period of about 1 year, we created 2,500 terms for coding our findings. This thesaurus was used as our dictionary. We picked variables thought to be clinically significant, but we also coded all minutiae concerning each case, realizing that it would be easy for vital information to be omitted from our current matrix. This would be like leaving out the *Anopheles* mosquito in a computerized study on the etiology of malaria.

A group of 224 cases, thought to be reasonably representative of reliable examples of spontaneous regression, was identified. Text-figure 1 presents a completed patient-data coding sheet, ready for keypunching, after we had transposed all information to standardized coding sheets. After verification, the material was then a part of the data base, ready for retrieval. Many of the observations, of course, were of a dichotomous or a yes/no nature. As originally conceived, the data were to assess 468 features or characteristics for each of the 224 patients. The constructed matrix if completely filled would have contained 104,832 observations. It is here, of course, that the problem of missing data emerges. Reported cases of spontaneous regression, as we all know, are generally lacking in any abundance of related information. In many original histories, for example, the examiner failed to record the definite presence or absence of many signs and symptoms. Thus we are left with an exceedingly sparse matrix of observations; only 6,229 observations were collected. This represents only 4.9% completion. Unfortunately, there is no statistical way to deal with missing data. Nevertheless, we wrote programs to examine the data that we had and to analyze our material, using the program called Gypsy (General Information Processing System) provided by Sweeney (4) of the University of Oklahoma. After the initial organization and editing of the data base were completed with this general system, better defined portions of the data base were selected, and particular analysis was performed by means of ad hoc Fortran programs. Our final matrix comprised 76 observations or combinations of observations from 224 patients, thus giving us the matrix structured with 17,024 separate squares. The hardware used consisted of a Digital Corporation PDP-12, an IBM 360-50, and other assorted pieces of equipment.

With the matrix reduced to slightly over 17,000 squares, the percentages of observations rose to 12%, a total of 2,024. With the use of a statistical analysis computer

AI (7)	UD	XA
H	V	XA4
HA (60)	VD	XB
HC (F)	VD4	XB2
K	VE	XC(5)
KM	VE2	
L	VF	
LD	VF1B	
LE	VF2	
LF	VF2B1	
LZ	VF3	
LZ4	VF3A	
LZ5	VF3A1	
M	VF3B	
MC4	VF3B2	
N	VF3C	
NP	VF3C1	
Q	VF3D	
QG	VF3D1	
QG2	VF3D2	
QG2C	VF3E	
S	VF3E1	
SA3	VF3F	
SA3A	VF3F2	
T	VT	
TI	W	
UB	WC	
	X	

TEXT-FIGURE 1.—Completed patient-data coding sheet, ready for keypunching.

program, we made 2X2 comparisons of the 224 patient reports to rank them in sequence, placing the most similar patient reports next to one another. The number of combinations for the 224 patients, taken 2 at a time, was 24,976. A frequency distribution of the number of differences between pairs showed very even distribution, about 10 differences between pairs. The final matrix assessed 16 clinical features for each of the 224 patients. To accomplish this, we combined certain features or categories empirically, but reasonably (for example, by grouping together all antimicrobial therapies). Because this final scheme contained no inappropriate coding terms, the matrix theoretically should have reflected 3,584 observations, 16X224. Actually, only 2,024+ observations were present. Hence the final matrix was 57% filled. Any truly rigorous computer-clustering technique was frustrated.

Much has been said about host resistance and the increasingly important position it is assuming. In this regard it may be that the spin-off from organ transplantation efforts will prove to be a most beneficial by-product, especially in providing a new perspective from which to view the phenomenon of spontaneous regression.

According to Dr. Israel Penn (personal communication), the instances in which cancer has appeared in the post-transplantation period now number 224 (the same number as our spontaneous regression cases). When there is interference with the normal surveillance mechanism for foreign cells, a group of wholly different types of cancer appears. Three of these have appeared in heart transplant patients: one from South Africa with carcinoma of the stomach (5); another from Colorado with carcinoma of the pancreas (Penn I: Personal communication); and one from St. Louis, who lived 8 months after receiving the transplant (William V: Personal communication) but died from extensive involvement of the heart and pericardium, probably from an unrecognized bronchogenic carcinoma in the donor.

In this connection, particular attention should be focused on the fact that there are instances of regression of the cancer when immunosuppressive activity is intentionally reduced, both in the patient with a de novo cancer and in those inadvertent recipients of cancer from the donor. Also, we might remind ourselves of the increased incidence of cancer among patients with severe immunologic de-

ficiencies, such as the Wiskott-Aldrich syndrome and ataxia-telangiectasia.

It would be highly salutary, I think, if we could computerize a profile on the typical patient with spontaneous regression. We know a few things. The cancers constituting approximately 63% of all in our group, i.e., of lung, breast, colon, rectum, pancreas, esophagus, stomach, and prostate, combine to represent only 12% of all the cases in our group undergoing spontaneous regression. On the other hand, over one-half (54%) of the spontaneous regressions are encountered for a small group of neoplasms constituting less than 4% of the total, i.e., melanoma, neuroblastoma, choriocarcinoma, and hypernephroma.

These latter four types of cancer to an extent, may influence the fact that spontaneous regression of cancer seems to be a phenomenon most likely to occur in the young patient. While 65% of the male patients experiencing spontaneous regression are in the 20-60 age group and most regression in females is seen before they reach the age of 50, we all recognize that the cancer problem, in general, predominates in the sixth and seventh decades of life. Is it the reflection of a more vigorous lymphoreticuloendothelial system in the earlier years? Are so-called host-resistant factors and hematologic surveillance mechanisms less competent as the body grows older? Certainly, there are many examples of apparent cancer regression in earlier years. We know that a malignant melanoma before puberty is unusual. Bolande (6) calls our attention to the remarkable frequency of neuroblastomas in situ in infants autopsied before they were 3 months of age. Clinically evident neuroblastomas spontaneously regressing have been observed repeatedly in infants younger than 1 year.

At the 1960 meeting of the Pan-Pacific Surgical Association, I presented a paper on maternal cancer, metastatic to the products of conception (7). Even though the focus is necessarily on the fact that it does occur, perhaps more importantly, we should focus on the fact that it is so rare in the hundreds of thousands of pregnancies complicated by carcinoma of the breast and cervix, melanoma, or whatever. A patient of mine with terminal metastatic melanoma, who had delivered an infant boy some 6-9 months earlier, came to our hospital, and as you may note in the histology of the placenta (fig. 2), there is both intravillous and intervillous tumor growth of placenta. Obviously, tumor cells were circulating in the cord blood. In spite of an enlarged liver and elevated alkaline phosphatase levels in the earlier months, the infant has remained well. Hoping that we might transfer an immune response to the infant's mother, we performed an exchange transfusion of this infant, but the mother, who was almost in extremis, died within 2 weeks.

Let me call your attention to the fact that this was the second of at least 2 bona fide cases of spontaneous regression involving cancer transferred from the mother to the fetus. Another is alive after regression of tumor in the

lung and leg (8). So the interesting aspect to me is not that maternal cancer metastatic to the products of conception has been reported 27 or 28 times in the world's literature since 1966 (9) but that it occurs so infrequently. What are the unique protective forces at work in the placenta or in the infant?

As we continue to probe, there may be many more examples of previously unrecognized spontaneous regression, some perhaps a bit subtle as in the "halo" melanoma or the unknown primary cancer. One message from this conference certainly seems indicated, and I think we all agree on this: Physicians must be encouraged to record their cases of possible spontaneous regression. In the course of discussion at several meetings this year, five physicians have told me about cases that they believe are spontaneous regressions, and I think there must be more lurking around. One doctor said he is reluctant to report his cases for fear of ridicule.

Finally, about 9 years ago, a young student was referred to me from the University Student Health Service with a melanoma on his back. We performed a wide resection and a subsequent axillary lymph node dissection. Two years later, scattered bilateral pulmonary metastases were seen, and knowing his prognosis, the young man continued in his work as a drama major. Eventually he invited my wife and me to what turned out to be his last performance in which, as fate would have it, he played the part of that delightful physician, Sir Calenso Ridgeon, in George Bernard Shaw's "The Doctor's Dilemma." In 1906 Ridgeon was obsessed with the need to "stimulate the phagocytes" as a universal therapeutic approach to disease. Almost 70 years later, the leukocytes and especially the lymphocytes are again near the center of the stage.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966
- (2) SUMNER WC: Spontaneous regression of malignant melanoma. Report of a case. *Cancer* 6:1040-1043, 1953
- (3) SUMNER WC, FORAKER AG: Spontaneous regression of human melanoma: Clinical and experimental studies. *Cancer* 13:79-81, 1960
- (4) SWEENEY: General Information Processing System. Part II. User's Guide. Norman, University of Oklahoma Office of Research Administration, 1973
- (5) STEPHENSON HE JR, DELMEZ JA, RENDEN DI, et al: Host immunity and spontaneous regression of cancer evaluated by computerized data reduction study. *Surg Gynecol Obstet* 133:649-655, 1971
- (6) BOLANDE RP: Benignity of neonatal tumors and concept of cancer regression in early life. *Am J Dis Child* 122:12-14, 1971
- (7) STEPHENSON HE JR: Transplacental metastasis of malignant tumor cells. *Audio-Digest Obstet Gynecol* 8: No. 9, May 1961
- (8) CAVEL B: Transplacental metastases of malignant melanoma. *Acta Paediatr Scand (suppl)* 146:37-40, 1963
- (9) STEPHENSON HE JR, TERRY CW, LUKENS JN, et al: Immunologic factors in human melanoma "metastatic" to products of gestation (with exchange transfusion of infant to mother). *Surgery* 69:515-522, 1971

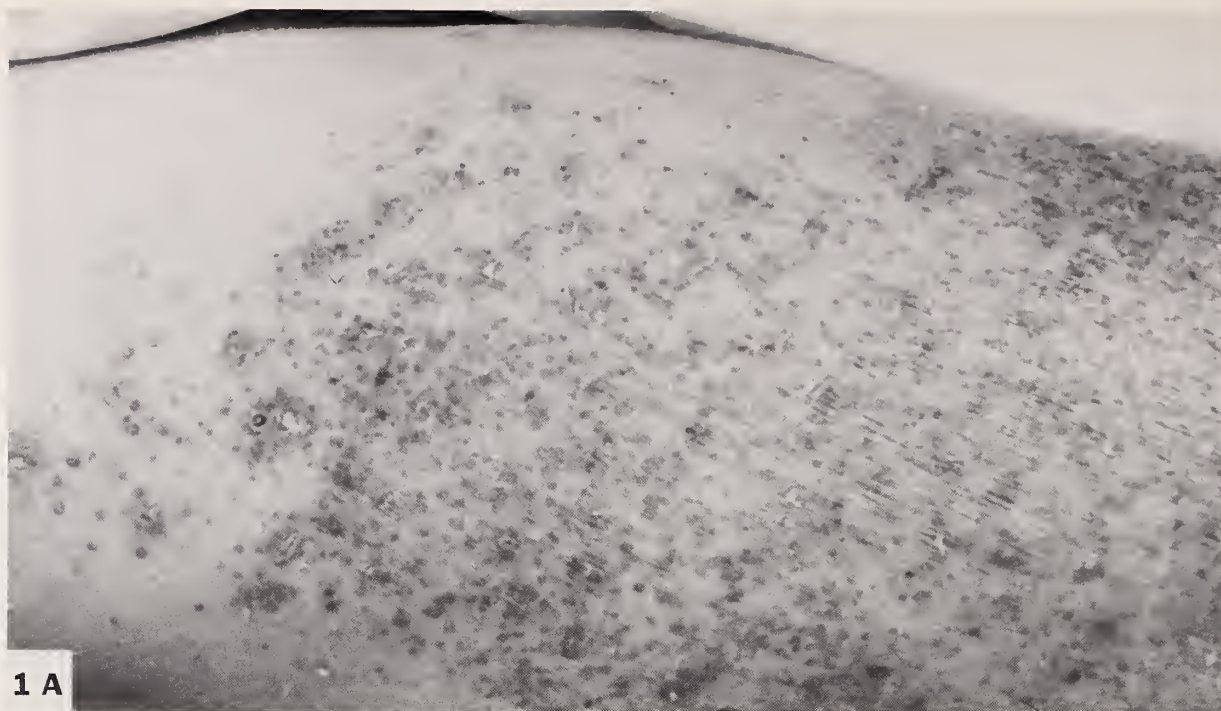


FIGURE 1.—A: Stasis melanosis or satellitosis of right thigh (4-2-71). B: Areas of vitiligo at site of previous melanotic lesions (11-23-71).

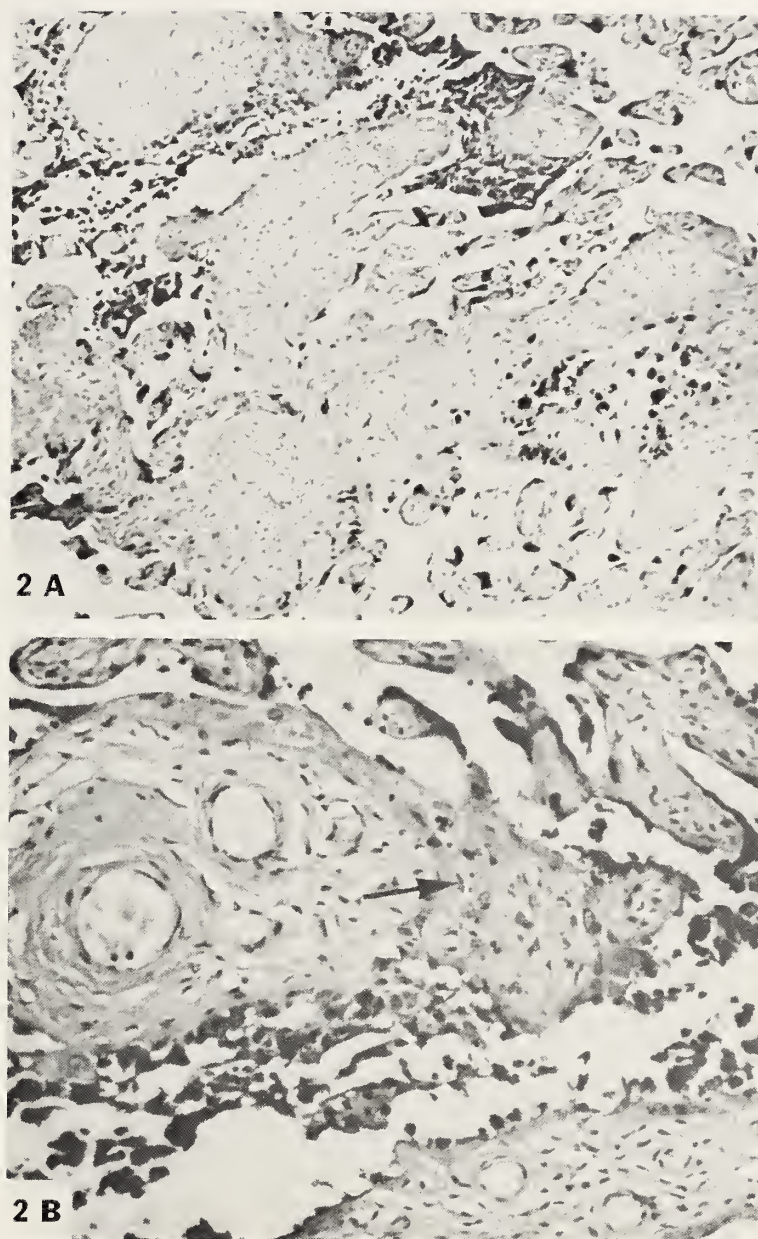


FIGURE 2.—A: Section of chorionic villi showing surrounding and invading melanoma cells. Hematoxylin and eosin (H & E). $\times 80$. B: Upper, left portion of the same chorionic villi section. *Arrow* indicates a point of invasion by pigmented melanoma cells. H & E. $\times 200$

Spontaneous Regression of Neuroblastoma^{1, 2}

Audrey E. Evans, James Gerson, and Louise Schnauffer^{3, 4}

SUMMARY—Cases of spontaneous regression of neuroblastoma continue to occur in the present multimodal therapy era at institutions where physicians are prepared to withhold treatment on certain patients with residual primary or metastatic disease. From a survey of the 22 member institutions of Children's Cancer Study Group, seven hospitals submitted data on 24 neuroblastoma patients whose disease underwent regression after minimal, unusual, or no treatment. An analysis of these patients and of 33 patients from two large series in the literature shows that the majority of patients are infants with Stage II or Stage IVS disease. The spontaneous regression usually consists of complete disappearance of the disease, but in some neuroblastomas, maturation to ganglioneuroma takes place. The various factors that may influence regression are discussed.—*Natl Cancer Inst Monogr* 44:49-54, 1976.

Neuroblastoma has long been cited as the prime example of a tumor that can undergo spontaneous regression. The comprehensive monograph by Everson and Cole (1) includes 29 case reports of neuroblastoma patients considered to have had true spontaneous regressions. These 29 form a considerable proportion (17%) of the total 176 patients included in the monograph. Considering the low incidence of neuroblastoma in the population, approximately 3 per million (2), it is striking that 17% of the case reports of spontaneous regression occur in this tumor. This fact suggests that there are unique characteristics of neuroblastoma. One of these is the ability to mature to a benign tumor. Although most instances of regression lead to complete disappearance of all evidence of tumor, there are cases, documented by histologic examinations, in which known malignant neuroblastoma becomes benign ganglioneuroma (3).

The definition of spontaneous regression is worthy of some discussion. The Everson and Cole monograph includes cases of patients who recovered 1) after no therapy, 2) after therapy that was directed at only part of the disease, and 3) after systemic therapy that by most standards would be considered nontherapeutic, such as arsenicals, or inadequate, such as one course of nitrogen mustard. Most of the following discussion will deal with the first two categories: completely untreated patients or those in whom some area of disease remained untreated.

CLINICAL DATA

The 22 member institutions of Children's Cancer Study Group (CCSG) were requested to share their experience with this phenomenon so that current information could be gathered regarding spontaneous regression. Seventeen institutions responded, including seven that submitted

information on patients whose disease regressed with little or no therapy.⁵ No attempt was made to calculate the base from which these patients were derived, because with the advances of present-day treatment of combined surgery, radiation, and chemotherapy, the incidence of spontaneous regression will depend more on the philosophy of the physician than on the vagaries of the disease. There are few pediatric oncologists who would feel justified in withholding systemic therapy confronted with residual or metastatic disease, in order to await possible spontaneous regression.

Not all cases submitted were included in the analysis, and we had some difficulty in selecting patients because some had received a form of therapy that by present day standards would be considered inadequate or inappropriate. All patients who received agents currently being used for the treatment of neuroblastoma, such as cyclophosphamide, vincristine, or nitrogen mustard, were excluded unless such treatment was very limited.

In an attempt to discern the type of patient who tends to exhibit spontaneous regression, we staged the disease according to the criteria of Evans et al. (4), as follows: 1) Stage I—disease confined to the organ or structure of origin; 2) Stage II—regional disease limited to one side of the midline; 3) Stage III—regional disease that crossed the midline; 4) Stage IV—hematogenous metastases to distant lymph nodes, skeleton, or organs; and 5) Stage IVS—hematogenous metastases limited to the liver, skin, and bone marrow, without evidence of skeletal involvement.

In addition to the study of the CCSG patients, a search of the literature was made to analyze other case reports of spontaneous regression of neuroblastoma.

RESULTS

The 24 patients reported can be roughly divided into two equal groups. Thirteen patients received no therapy other than incomplete resection of the primary tumor, or they had areas of metastatic disease that were untreated; 11 patients received systemic therapy that we have termed minimal or unusual. Many of the patients, including most of those from the Children's Hospital of Philadelphia, have been reported previously (5).

The clinical data for the 13 patients with some tumor untreated are given in table 1.

Two cases are detailed below, since they clearly illustrate the two common types of spontaneous regression, i.e., the complete disappearance of all evidence of disease and the maturation of malignant neuroblastoma to benign ganglioneuroma.

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³ Children's Hospital of Philadelphia, 34th and Civic Center Boulevard, Philadelphia, Pa. 19104. For Children's Cancer Study Group.

⁴ We acknowledge the thoughtful contributions of Dr. G. J. D'Angio to this study.

⁵ Columbus Children's Hospital, Columbus, Ohio (William A. Newton, Jr.); Children's Memorial Hospital, Chicago, Ill. (Alan Schwartz); University of Minnesota, Minneapolis, Minn. (Mark Nesbit); Babies Hospital, New York, N.Y. (James A. Wolff); University of California, San Francisco, Calif. (Arthur R. Ablin); Children's Hospital of Philadelphia, Philadelphia, Pa. (Audrey E. Evans); and Children's Hospital of Los Angeles, Los Angeles, Calif. (Daniel M. Hayes).

TABLE 1.—*Spontaneous regression of neuroblastoma in 13 CCSG patients*

Case No.	Age (mo)	Sex	Stage at diagnosis	Primary site	Site of metastasis	Treatment to primary ^a	Treatment to metastases ^a	Survival (yr)
1 ^b	1	F	II	Thorax + extradural	—	Partial surgery	—	9
2 ^b	36	F	II	Thorax	—	Partial surgery	—	8
3 ^b	30	F	II	Thorax	—	Partial surgery	—	10
4 ^c	10	F	II	Thorax + extradural	—	Partial surgery	—	9
5 ^b	18	F	III	Abdomen	—	Partial surgery	—	16
6 ^b	2	M	IVS	Abdomen	Liver, ? skin	None	None	21
7 ^b	2	F	IVS	Abdomen	Liver	None	None	22
8 ^b	4	F	IVS	Left adrenal	Liver, marrow	Excision	None	8
9 ^b	5	M	IVS	Right adrenal	Liver	Partial surgery	None	8
10 ^d	18	F	IVS	Unknown	Liver	None	None	2
11 ^d	2	F	IVS	Abdomen	Skin	None	None	2
12 ^b	56	M	IV	Thorax, abdomen	Nodes, marrow	Partial surgery	None	13
13 ^e	72	F	IV	Thorax	Nodes, bones	XRT	XRT to some bone lesions	14

^a XRT=X-ray therapy.^b Children's Hospital of Philadelphia.^c Children's Hospital of Los Angeles.^d Children's Memorial Hospital, Chicago.^e University of California, San Francisco.

Case History No. 1.—A newborn girl was seen in 1970 at 3 days of age because of a large abdominal mass. Abdominal exploration showed the mass to be caused by a grossly enlarged liver infiltrated with tumor which proved to be neuroblastoma. No evidence of a primary neoplasm was found at that time. No treatment was given, and the liver gradually decreased in size over a period of 10 months. The baby was reexplored at the age of 1 year, and no evidence of tumor was found in the liver. A small mass was palpated in the left adrenal, which was removed and found to contain a small neuroblastoma. Again, no treatment was given, and the child remains well 3 years later. [This patient is not included in the CCSG statistics, since she was treated by one of us (L.S.) and at another institution.]

Case History No. 2.—A 4-year-old male was referred for treatment in 1960, after a biopsy of a large abdominal mass showed ganglioneuroma. In addition, there was a lesion in the superior-posterior mediastinum. The mediastinal mass was completely resected, and it too was a ganglioneuroma. A regional lymph node contained ganglioneuroma. A bone marrow aspiration revealed tumor cells in clusters of 5–15 cells.

One month later the large retroperitoneal mass was incompletely resected and proved to be ganglioneuroma without evidence of neuroblastoma. Five years later a needle biopsy of the residual abdominal mass again showed ganglioneuroma. The patient has remained asymptomatic for 14 years. Although ganglioneuroma is a benign tumor of the sympathetic nervous system, its presence in a lymph node and the bone marrow suggests that it originated as a malignant neuroblastoma (*see below*).

The 11 patients who received minimal or unusual treatment are listed in table 2, in order of increasingly aggressive therapy. This group cannot be considered as having "true" spontaneous regression. It is included for completeness of data, and one must make one's own judgment about whether therapy or some characteristic of the patient is the more likely cause of the fortunate result.

In an attempt to determine whether a particular patient is likely to recover with minimal or no therapy, tables 1 and 2 are combined and summarized in table 3, according to the stage of disease and age of patient at diagnosis. It is known that the patient's age at diagnosis is related inversely to the prognosis; the younger the patient, the better the chance of survival. It has also been shown that age of patient and stage of disease are independent variables (5).

There are two major reports in the literature dealing with a moderately large number of patients with neuroblastoma who exhibited spontaneous regression after no therapy or after treatment with agents not considered by most investigators to be cancer chemotherapy, such as vitamin B₁₂ or bacterial toxins. These reports are by Everson and Cole (1) and by Bodian (6). The Everson and Cole monograph includes some patients from Bodian's article and from the Children's Hospital of Philadelphia, so there is a certain amount of duplication. Not all the patients in the Bodian article were included because the authors believed the local measures used could have been sufficient to cure the patient. The patient data from these two reports are given in tables 4 and 5. There was difficulty in staging the disease of some patients because sufficient data regarding the extent of the primary tumor were not always given.

Finally, all data of the 57 patients included in tables 1, 2, 4, and 5 (avoiding duplication) are summarized to form table 6. The patients have been divided into two groups: 1) those who had residual or metastatic disease that was untreated and can thus be classified as exhibiting spontaneous regression by any criteria, and 2) those who had received some form of therapy directed to all sites.

Of the 24 patients listed by the CCSG institutions, 13 are from the Children's Hospital of Philadelphia. This proportion is high because for many years Dr. Koop (7) believed that vigorous treatment was not necessary in every case of neuroblastoma with residual disease and might even, in some cases, be harmful. Therefore, it was not unusual for some patients with minimal residual disease not to

TABLE 2.—Regression of neuroblastoma in 11 CCSG patients following minimal or unusual treatment

Case No.	Age (mo)	Sex	Stage at diagnosis	Primary site	Site of metastasis	Treatment to primary	Treatment to metastases ^a	Survival (yr)
1 ^b	1	F	IVS	Abdomen	Skin	None	FUDR	10
2 ^b	2	F	IVS	Abdomen	Liver	None	Amino-an-fol	10
3 ^b	9	F	IVS	Abdomen	Liver	Partial resection	Amino-an-fol	14
4 ^c	9	F	IV	Chest	Skin	XRT, 1,400 R	Vitamin B ₁₂	9
5 ^d	5	M	IVS	Abdomen	Skin, liver, marrow.	XRT, 2,500 R	XRT to liver	?
6 ^e	3	F	IVS	Unknown	Skin, liver	—	XRT to liver, 3,000 R; 6-MP + prednisone	16
7 ^f	2	F	IVS	Abdomen	Skin, liver	XRT, 200 R	XRT to liver, 2,000 R; HN ₂ × 2 courses	18
8 ^g	120	F	IV	Unknown	Bones	Unknown	XRT, 700 R, vitamin B ₁₂	10
9 ^d	1	M	IVS	Abdomen	Skin	XRT, 900 R	Aminopterin, TEM, AMD × 2	17
10 ^h	2	M	IVS	Adrenal	Liver	Complete resection	XRT, 900 R	6
11 ^b	2	F	IVS	Abdomen	Liver	XRT, 800 R	XRT, 800 R; CPM, 2 doses	1

^a FUDR=2'-fluoro-5-deoxyuridine; 6-MP=6-mercaptopurine; HN₂=nitrogen mustard; TEM=triethylenemelamine; AMD=daunomycin; and CPM=cyclophosphamide.

^b Children's Hospital of Philadelphia.

^c University of Minnesota.

^d Columbus Children's Hospital.

^e Babies Hospital, New York.

^f Children's Hospital of Los Angeles.

^g University of California, San Francisco.

^h Children's Memorial Hospital in Chicago.

TABLE 3.—Regression of neuroblastoma in 24 CCSG patients following minimal, unusual, or no treatment^a

Age of patient (mo)	Stage of disease				Total
	II	III	IV	IVS	
0-5	1	—	—	14	15
6-11	1	—	1	1	3
12-23	—	1	—	—	1
24+	2	—	3	—	5
Total	4	1	4	15	24

^aNo treatment means that significant residual neuroblastoma remained untreated.

receive postoperative irradiation or chemotherapy. The 13 cases of spontaneous regression are derived from a total of 174 neuroblastoma patients, giving a rate of 8%.

DISCUSSION

The results from the data submitted by the CCSG institutions reinforce the fact that regression of a considerable mass of neuroblastoma occasionally occurs without any specific therapy. The experience at Children's Memorial Hospital in Chicago is worth reiterating (8). In a period of 18 months, Schwartz and his colleagues saw 3 infants with Stage IVS neuroblastoma, with liver or skin metastases. The first patient was vigorously treated with chemotherapy and radiotherapy, which resulted in no obvious clinical response; in fact, for a time the tumor continued to grow, and it was concluded that the treatment was ineffective. Subsequently the disease regressed, and the child remains well with no further treatment. (Many case reports have shown that the tumors continued to grow initially despite therapy, which was then abandoned.) Because of their

experience with the first patient with Stage IVS disease, the pediatricians at Children's Memorial Hospital elected not to treat the next 2 patients they encountered; both children are at present doing well with no evidence of disease (cases #10 and 11, table 1).

What type of patient with neuroblastoma is most likely to undergo spontaneous regression? All stages appear to be affected, although Stage I patients cannot be evaluated because the disease, being confined to the organ of origin, is almost always totally resected. However, patients in Stages II and IVS provide the majority of regressions, i.e., 32 and 39%, respectively (table 6). All the patients in Stage II had partial removal of the primary tumor followed by regression of the remainder of the tumor. The patients in Stage IVS are more interesting because they have a much larger quantity of residual tumor, which in the majority of patients involves the liver, skin, or both. In only 3 of 28 patients with Stage IVS disease was there tumor in the bone marrow. Lastly, all patients are young; the majority are infants a few months of age with a small or undetected primary tumor and with widespread disease in the skin, liver, or both.

Factors Influencing Spontaneous Regression

Mechanical.—It has been proposed that partial resection of the tumor, hemorrhage, or interruption to the blood supply cause tissue necrosis and death of residual tumor. This conjecture could be the explanation in some cases of regional disease but could hardly be the cause of regression in sites distant from the primary tumor. Also, it does not seem to occur in other pediatric tumors.

Age.—The young age of these patients is striking; 65% were seen before their sixth month. One could postulate that early in life the balance between host defense mechanisms and blocking antibodies favors the patient. Carry-over of the maternal immune factors would not seem an

TABLE 4.—*Clinical data, therapy, and survival of 29 patients with neuroblastoma from (1)*

Case No.	Age (mo) ^a	Sex	Primary site	Stage	Site of metastasis	Treatment to primary	Treatment to metastases	Survival (yr)
1	20	M	Thorax + extra-dural	II	None	Coley's toxins (16 mo)	—	46 ^b
2	7	F	Abdomen	IVS	Liver, skin	XRT × 7 days	XRT + liver, skin 0	7
3	NB	M	Unknown	IVS	?Liver, skin	—	Arsenical drugs	3
4	NB	F	Thorax	IVS	Skin, marrow	XRT	XRT, some skin	3
5	4	M	Unknown	IVS	Skin, scrotum	—	None	6
6	NB	F	Abdomen	I or II	None	Biopsy; XRT × 3	—	5
7	6	M	Abdomen	IV	Liver, bone	Biopsy; XRT, 1,200 R	None	2
8	7	M	Left adrenal	IV	Bone,	Primary resected	Corticosteroids (10 days)	2
9	NB	F	Unknown	IVS	Liver, skin	—	HN ₂ × 5 yr	9 ^b
10	4	M	Abdomen	IVS	Skin	Secondary resection	Coley's toxins	15 ^b
11	11	F	Thorax	II	—	Biopsy	—	9
12	1	M	Pelvis	II	—	Biopsy	—	19
13	4	M	Right adrenal	IV	Liver, skin, bone	Biopsy	TEM × 3 days	4
14	24	F	Thorax	II	Cervix	Biopsy	None	6
15	NB	M	Unknown	IVS	Liver, skin,	—	None	2
16	17	F	Abdomen, celiac axis	III	None	Partial resection	—	7
17	2	M	Abdomen	IVS	Liver, skin	Partial resection	None	10
18	2	M	Unknown	IVS	Liver	Biopsy	None	14
19	8	F	Right adrenal	IVS	Liver	Primary resection	None	7
20	1	M	Thorax	II	None	Partial resection	—	6
21	2	F	Unknown	IVS	Liver	Biopsy	None	7
22	6	F	Thorax + extra-dural	II	Cervical nodes	Biopsy	—	15
23	?5	M	Cervix	II	None	None	—	4
24	9	F	Right adrenal	II	Regional nodes	None	Partial resection of nodes	12
25	4	M	Thorax	IVS	Liver, skin	None	None	11
26	1	F	Right adrenal	IVS	Skin	None	XRT, 2,400 R to one lesion; prednisone	2
27	NB	F	Left adrenal	IVS	Skin, nodes	None	Prednisone (14 days)	7
28	3	F	Pelvis + extra-dural	IVS	Skin	Biopsy	None	9 ^b
29	1	M	Unknown	IVS	Skin	—	XRT, 700 R to some skin lesions	16 ^b

^a NB=newborn.^b Maturation to ganglioneuroma.

adequate explanation. The tumor should have been destroyed in utero or, if maternal influences were important, should never have developed. The immune response to neuroblastoma is discussed in greater detail below.

Another clue to the influence of age is available from the reports of Beckwith and Perrin (9) and Guin et al. (10). In 1963, Beckwith and Perrin reported the presence of so-called in situ neuroblastoma in the adrenals removed at autopsy from 13 infants who died of diseases unrelated to cancer. The authors calculated that the incidence was 1:100 to 1:300 infant autopsies, a 2 to 3 order of magnitude figure, higher than the annual incidence of neuroblastoma, which is approximately 1:300,000 children. The report by Guin et al. on "incidental" neuroblastoma in infants includes 5 of 11 patients whose tumors were larger than the in situ category. Again, in spite of a diligent search, all were found in infants 3 months or younger and none were

found in older infants. In their report, the frequency of incidental neuroblastoma increased over the years 1944-67, probably because of increasing awareness of subclinical neuroblastoma. The incidence increased from 1:635 to 1:39 (4 cases in 155 autopsies).

There is some disagreement about whether in situ neuroblastoma is a true precursor of the malignant tumor or whether it is a histologic variation of normal infant adrenal cortex. There is no disagreement, however, that the lesion seen in infants during the first 3 months of life disappears.

Site of disease.—Primary: The site of the primary cancer does not appear to be the crucial factor. All areas of the sympathetic nervous system are represented in patients with spontaneous regression. The size of the primary, however, may be important; most patients with local residual disease that regressed were in Stage II, and many of those in Stage IVS had small or undetected primaries.

TABLE 5.—Regression of neuroblastoma in 11 patients following treatment with vitamin B₁₂ (6)

Case No.	Age (mo)	Sex	Stage	Primary site	Site of metastasis	Treatment to primary	Treatment to metastasis	Survival (yr)
1	7	M	III	Pelvis	None	Vitamin B ₁₂	—	12
3	5	F	II	Cervix	Local recurrence	XRT, 400 R	Vitamin B ₁₂ (recurrence)	9
4	10	F	II	Abdomen + extradural	None	Partial excision; vitamin B ₁₂	—	2
5	4	F	II	Abdomen + extradural	None	Biopsy; vitamin B ₁₂	—	3
8	NB	F	IVS	Unknown	Liver	—	Vitamin B ₁₂	7
9	12	F	IV	Left adrenal	Lymph nodes, subcutaneous	Excision	Vitamin B ₁₂	7
11	NB	M	IV	Abdomen + extradural	Bone	Biopsy	Vitamin B ₁₂	6
12	3	F	?II	Pelvis	—	Biopsy; vitamin B ₁₂	—	8
14	<1	F	?II	Pelvis	—	Partial resection; vitamin B ₁₂	—	
19	5	F	IVS	Unknown	Liver	—	Vitamin B ₁₂	4
29	4	F	IVS	Left adrenal	Skin, liver	Excision	XRT, 850 R to liver; vitamin B ₁₂	2

TABLE 6.—Regression of neuroblastoma in 57 patients after minimal, unusual, or no treatment

Age (mo)	Stage				Total
	II	III	IV	IVS	
Minimal or unusual treatment					
0-5	4	—	2	13	19
6-11	1	1	2	—	4
12-23	1	—	1	—	2
24+	—	—	1	—	1
Total	6	1	6	13	26
No treatment ^a					
0-5	5	—	—	13	18
6-11	4	—	1	2	7
12-23	—	1	—	—	1
24+	3	—	2	—	5
Total	12	1	3	15	31

*Significant residual neuroblastoma remained untreated.

Metastases: The sites of dissemination appear to have a great influence on spontaneous regression. Of 37 patients with disseminated disease, 28 had lesions in the liver, skin, or both. Only 9 of the 37 had X-ray evidence of bone metastases, which is a low rate compared with the incidence of such sites of involvement in the neuroblastoma population as a whole. Dissemination to the skin is almost unique, because practically all patients who exhibit this type of spread survive. Again, one might invoke an immune mechanism with subcutaneous tumor cells stimulating the

regional lymph nodes and leading to a particularly effective immune response. Interestingly, melanoma provides the third largest group of tumors (19 of 176) undergoing spontaneous regression in the patients summarized by Everson and Cole (1).

The unusual response of metastases in certain sites, e.g., skin and liver, might be explained alternatively by designating these neoplasms as multiple primaries rather than metastases from a single primary (11). In the early stages of embryonic development, the nervous system arises from ectoderm. Neural crest cells then migrate and give rise to many different types of cells in the embryo. Some migrate to the area of the dorsal mesentery to give rise to the autonomic ganglion cells; others from melanocytes and Schwann cells in the skin. Neuroblastoma localized to the skin could be either neural crest cells that were unspecified at an early stage or autonomic ganglion cells that followed an aberrant migratory pathway.

The same hypothesis can be made about the liver with cells from the dorsal mesentery infiltrating the liver itself, along with autonomic cells going to adjacent structures. However, there is no specific neural crest derivative for the liver as there is for the skin. The conceptual difference between these lesions being multiple primaries rather than metastases is that the presence of metastases implies a poor immune defense. Conversely, multiple primaries might be more antigenic and better rejected by the host. Regression of the metastases in the liver is perhaps even more remarkable than that in the skin. Some infants have massive amounts of tumor in the liver which tend to be diffusely involved rather than nodular. It is difficult to invoke a mechanism for spontaneous regression in this organ unless some aspect of the liver such as its reticuloendothelial system or its double blood supply assists in the rejection. This does not happen, however, with other secondary tumors in the liver. The other organ with a double blood supply, the lung, is rarely the site of metastatic neuroblastoma, but neither is it known to be an area in which spontaneous regression occurs.

Host Defense Mechanisms

Much of the recent work on the role of host defenses in control of cancer has been based on studies of patients with neuroblastoma. The incidence of spontaneous regression is cited as a possible example of an immune response. Hellström et al. found that lymphocytes from children with neuroblastoma inhibit the formation of colonies of neuroblastoma cells in culture, but not normal fibroblasts or cells derived from other tumor types (12, 13). Indeed, lymphocytes from all patients with neuroblastoma are cytotoxic to cultures of any neuroblastoma tumor, and this effect is not related to the extent of disease. They have also shown that the lymphocytes of the mothers of neuroblastoma patients are cytotoxic to the same tumor culture (12). It is presumed that this reaction depends on tumor-associated antigens.

Unfortunately, the presence of cytotoxic lymphocytes in patients with neuroblastoma does not insure a satisfactory outcome. The extensive work of the Hellströms et al. (12-14) has demonstrated that the sera of many patients with neuroblastoma contain a factor that prevents the effect of the immune lymphocyte against the target cell. Current evidence suggests these blocking factors are antigen-antibody complexes (15). A recent article by José and Skvaril (16) has shown that the blocking factor(s) can be identified by the use of immunoabsorbents and that IgG 1, 3, and 4 were involved. Also, the presence of blocking factors reflects the response of the patient, with decreasing levels after removal of the primary tumor and increasing amounts in patients with progressive disease. The presence of these factors may be one mechanism whereby tumors can grow despite a normal cell-mediated reaction to the tumor.

Thus the better survival experience observed in infants could be explained by an efficient immune response. The lymphocyte count in infants is higher than in older children, and there is evidence that the presence of lymphocytes in the peripheral blood, in the bone marrow, and in tumors correlates with a better prognosis (17-19). Perhaps these are T lymphocytes, as suggested by the blastic appearance of the cells in the bone marrow, and are thus cytotoxic. It is not possible to say that tumor-host interactions are the cause of better survival experience in infants or in patients with Stage IVS disease, but the hypothesis is attractive.

Maturation

Though not truly a spontaneous regression, maturation of the malignant neuroblastoma cell to a benign ganglion cell is extraordinary and is worthy of inclusion here.

In some cases the primary tumor or metastasis matures to a ganglioneuroma. It has been argued that maturation does not occur and that such cases are instances of residual ganglioneuroma cells persisting after the total disappearance of the neuroblastoma cells in a mixed ganglioneuroblastoma. There are conceptual arguments against such a hypothesis, but the conclusive evidence is the presence of benign ganglioneuroma in the regional nodes draining a neuroblastoma. Such a patient is depicted in the second case history given above. Maturation of neuroblastoma to ganglioneuroma is the only satisfactory explanation for this phenomenon; the concept of benign metastasizing ganglioneuroma is an absurdity.

Very little is known about control of nerve cell differentiation in the human. In neuroblastoma tissue cultures, differentiation can be induced by various means such as nerve growth factor, serum deprivation, and the addition

of cyclic nucleotides (20-23). However, the actual mechanism of human neuroblastoma maturation remains a matter of speculation. Finally, there are rare instances of what appears to be recrudescence of disease in sites having undergone spontaneous regression, maturation, or both. The ease reported by Griff and Griff (24) is an example. This 3-year-old girl had a posterior mediastinal mass, revealed by X-ray, which waxed and waned in size for 5 years until it was removed and found to be a "maturing" neuroblastoma 8×8×4 cm in size.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966, pp 11-87
- (2) SUTOW WW, VIETTI TJ, FERNBACK DI: Clinical Pediatric Oncology. St. Louis, Mosby, 1973, p 2
- (3) CUSHING H, WOLBACH SB: The transformation of a malignant paravertebral sympathicoblastoma into a benign ganglioneuroma. *Am J Pathol* 3:203-216, 1927
- (4) EVANS AE, D'ANGIO GJ, RANDOLPH J: A proposed staging for children with neuroblastoma. *Cancer* 27:324, 1971
- (5) BRESLOW H, MCCANN B: Statistical estimation of prognosis for children with neuroblastoma. *Cancer Res* 31:2098-2103, 1971
- (6) BODIAN M: Neuroblastoma: An evaluation of its natural history and the effects of therapy with particular reference to treatment by massive doses of vitamin B₁₂. *Arch Dis Child* 38:606, 1963
- (7) KOOP CE: The neuroblastoma. In *Progress in Pediatric Surgery* (Rickham PP, Hecker WC, Prevot J, eds.), vol 4. Baltimore, Univ Park Press, 1972, pp 1-28
- (8) SCHWARTZ A, DADASH-ZEDEH M, LEE H, et al: Spontaneous regression of disseminated neuroblastoma. *J Pediatr* 85:760-763, 1974
- (9) BECKWITH JB, PERRIN EV: In situ neuroblastomas: A contribution to the natural history of neural crest tumors. *Am J Pathol* 43:1089-1100, 1963
- (10) GUIN G, GILBERT E, JONES B: Incidental neuroblastoma in infants. *Am J Clin Pathol* 51:126-136, 1969
- (11) D'ANGIO GJ, LYSER KM, URUNAY G: Neuroblastoma, Stage IV-S: A special entity? Memorial Sloan-Kettering Cancer Center Clinical Bulletin 2 (No. 2): 61-65, 1971
- (12) HELLSTRÖM I, HELLSTRÖM KE, BILL AH, et al: Studies on cellular immunity to human neuroblastoma cells. *Int J Cancer* 6:172-188, 1970
- (13) HELLSTRÖM I, HELLSTRÖM KE, PIERCE GE, et al: Demonstration of cell bound and humoral immunity against neuroblastoma cells. *Proc Natl Acad Sci USA* 60:1231-1238, 1968
- (14) HELLSTRÖM I, SJÖGREN HO, WARNER G, et al: Blocking of cell-mediated tumour immunity by sera from patients with growing neoplasms. *Int J Cancer* 7:226-237, 1971
- (15) SJÖGREN HO, HELLSTRÖM I, BANSAL SC, et al: Suggestive evidence that the "blocking antibodies" of tumor-bearing individuals may be antigen-antibody complexes. *Proc Natl Acad Sci USA* 68:1372-1375, 1971
- (16) JOSÉ G, SKVARIL F: Serum inhibitors of cellular immunity in human neuroblastoma IgG subclass of blocking activity. *Int J Cancer* 13:173-178, 1974
- (17) BILL AH, MORGAN A: Evidence for immune reactions to neuroblastoma and future possibilities for investigations. *J Pediatr Surg* 5:111-116, 1970
- (18) EVANS AE, HUMMELER K: The significance of primitive cells in marrow aspirates of children with neuroblastoma. *Cancer* 32:906, 1973
- (19) BECKWITH JB, MARTIN RG: Observations on the histopathology of neuroblastoma. *J Pediatr Surg* 3:106-110, 1968
- (20) BURDMAN JA, GOLDSTEIN MH: Long-term tissue culture of neuroblastoma. III. In vitro studies of a nerve growth-stimulating factor in sera of children with neuroblastoma. *J Natl Cancer Inst* 33:123-133, 1964
- (21) SHUBERT D, HUMPHREY S, VITRY F, et al: Induced differentiation of a neuroblastoma. *Dev Biol* 25:514-546, 1976
- (22) PRASAD KN, SHEPPARD JR: Neuroblastoma cell culture: Membrane changes during cyclic AMP-induced morphological differentiation. *Proc Soc Exp Biol Med* 141:240, 1972
- (23) PRASAD KN, KUMAR S, GILMER K, et al: Cyclic AMP-induced differentiated neuroblastoma cells: Changes in total nucleic acid and protein contents. *Biochem Biophys Res Commun* 50:973-977, 1973
- (24) GRIFF LC, GRIFF RE: Neuroblastoma—Emphasis on the mediastinal neuroblastoma. *Am J Roentgenol Radium Ther Nucl Med* 103:19, 1968

Spontaneous Regression of Sarcoma^{1, 2}

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SUMMARY—Three pathologic processes generally regarded as nonneoplastic conditions are presented and discussed as examples of poorly controlled proliferations of lymphoid tissue. A study of these three disorders (infectious mononucleosis, hyperthyroidism, and Dilantin-induced lymphadenopathy) provides clues to an understanding of the pathogenesis and occasional spontaneous regression of the malignant lymphomas.—*Natl Cancer Inst Monogr* 44: 55-57, 1976.

Eleven cases of spontaneous regression of soft tissue sarcoma were reported by Everson and Cole (1) in a 1966 monograph. The details of these regressions are clearly presented in the monograph and will not be reviewed here. I have been unable to find valid reported examples of spontaneously regressing soft tissue sarcomas since the publication of Everson and Cole's review and, therefore, will discuss the regression of sarcoma-like disorders.

Implicit in the term "spontaneous regression of cancer" is the assumption that malignant tumors are composed of autonomous cells which, by definition, are independent of homeostatic control mechanisms. Both recent and not so recent clinical observations suggest that this assumption may not be valid, at least in discussions of the mesenchymal tumors included under the umbrella term "sarcoma," with specific reference to lymphosarcoma or malignant lymphoma. Unexplained regression of lymphadenopathy is not surprising when experience accustoms us to expect it. Lymphadenopathy in association with the viral exanthems or with infectious mononucleosis regularly regresses, although the specific mechanism of this regression has not yet been elucidated. What is the basis for the assumed qualitative difference between these reactive lymph nodes that regularly regress and those of malignant lymphoma that regress only rarely in the absence of iatrogenic intervention?

MALIGNANT LYMPHOMA

In most cases the inducing agent believed responsible for self-limited reactive lymphadenopathy is known, whether it is measles virus, varicella, or the herpes-like virus presumed to have a causative role in the pathogenesis of infectious mononucleosis. In human malignant lymphoma, no such causative agent has yet been reproducibly identified. Laboratory studies have, however, helped define an environ-

ment conducive to the development of malignant lymphoma.

A proposed model for the pathogenesis of experimentally induced lymphomas is illustrated in text-figure 1. This model shows regulation of the immune response through feedback control by antibody that can turn the system off, probably by binding to the antigen. In addition, there apparently exists a subpopulation of T cells that can suppress the immune response. Presumably these suppressor T cells act on B cells through recognition of a neoantigen that appears on the surface of a stimulated B cell. Although their exact mode of suppressor activity is not clear, the phenomenology is very clear, namely, that the immune response can be turned off, under appropriate conditions, by a subject's being supplied with T cells.

It is not terribly difficult to envision how an inhibitor of T-lymphocyte activity, administered in the presence of continued B-cell stimulation, could, by releasing the B-cell response from T-cell suppression, allow physiologic B-cell proliferation to overwhelm the suppressed control arm. The resulting clinical picture is malignant lymphoma, although there need be no intrinsic autonomy to the responding lymphocytes that make up the tumor.

INFECTIOUS MONONUCLEOSIS

Of course, the major responsibility for determining benignity or malignancy of a lymph node biopsy material rests on the anatomist who uses experience-proven criteria to differentiate benign from malignant disease. In this context, a review of 11 lymph node biopsies performed at the Mayo Clinic and reported by Salvador et al. (2) is particularly relevant. The histologic features observed in these lymph nodes from 11 different patients with infectious mononucleosis are listed in table 1.

Distortion or partial effacement of the normal lymph node architecture was observed in all 11 biopsies. Infiltration of the lymph node capsule by mononuclear cells with extension to perinodal fat was also noted in all specimens. Additional features observed in most or all biopsies included a high mitotic index with up to 10 mitotic figures present per high-power field, focal obliteration or total effacement of the subcapsular sinus, and the presence of bizarre-appearing histiocytes, now more appropriately called reticular lymphoblasts (3) (resembling the Reed-Sternberg cell of Hodgkin's disease). All these histologic findings are characteristic of malignant lymphoma, and yet all 11 patients in this report had peripheral smears reportedly consistent with a diagnosis of infectious mononucleosis; all had an absorbed heterophile titer greater than 1:224 and 10 of the 11 had titers greater than 1:448; all 11 had spontaneous regression of the lymphadenopathy and have remained well during a follow-up period lasting, for all but 2, from 2½ to 13 years.

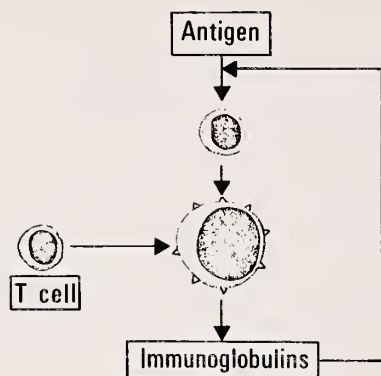
An experienced lymphopathologist can, I believe, differentiate these biopsy specimens of mononucleosis-affected lymph nodes from those of malignant lymphoma; never-

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TEXT-FIGURE 1.—Proposed model for regulation of immune reactivity through antibody feedback and suppressor T-cell activity.

TABLE 1.—*Lymph node histology in infectious mononucleosis* (2)

Histology	Number of patients (%)
Distortion or partial effacement of the normal lymph node architecture	11(100)
Infiltration of the lymph node capsule by mononuclear cells with perinodal extension	11(100)
Marked mitotic activity	11(100)
Focal obliteration of the subcapsular sinus	Most
Reticular lymphoblasts (Reed-Sternberg cells?)	11(100)

theless, the strong morphologic similarity of these two disease processes when considered in the context of defective T-cell regulation of B-cell reactivity, already proposed as a possible pathogenesis for malignant lymphoma, suggests that infectious mononucleosis is a lymphoma that regularly undergoes spontaneous regression.

In support of this concept, there is considerable current data relevant to infectious mononucleosis. A causal role for the Epstein-Barr virus has been strongly suggested (4). Pattengale and his co-workers (5) demonstrated that this virus, at least in culture, is capable of infecting and stimulating only those lymphocytes with immunoglobulin receptors on their cell membranes, namely B cells. Adams and his associates (6) reported collecting cells from a Hodgkin's disease-involved lymph node and intra-peritoneally injecting them into an immunosuppressed hamster. The injected cells gave rise to a metastatic, human immunoglobulin-secreting lymphoma that killed most of the recipient hamsters within 2 weeks. When the same experiment was repeated, with peripheral blood from patients with infectious mononucleosis in place of the Hodgkin's disease lymph node, a strikingly similar picture resulted, namely, a metastatic B-cell or human immunoglobulin-secreting lymphoma developed that killed most of the recipient hamsters within 2 weeks.

Bar et al. (7) recently reported 4 cases of fatal infectious mononucleosis in a single kindred. They found Epstein-Barr virus in the lymph node cells of the 1 patient studied, 26% atypical lymphocytes on peripheral smear, an absorbed heterophile titer of 1:224, a marked polyclonal increase of circulating B cells, and postmortem evidence of lymphocyte and plasmacytoid lymphocyte infiltration of small bowel, liver, and brain. The preponderance of B cells in this case, manifested both in the peripheral blood

and at autopsy, contrasts with the more common observation of increased peripheral blood T cells in this disorder (8). All these data are consistent with the concept of infectious mononucleosis as a proliferation of B cells in response to antigenic stimulation, which is eventually controlled by T-cell regulation. The transient depression of regulator T-cell function, which allows this disease to appear, is evidenced both by tuberculin anergy during infectious mononucleosis, as reported by Haider et al. (9) for 5 of 8 patients who had been previously vaccinated with BCG, and by the development of a medley of inappropriate antibodies reported appearing transiently during the course of this disease (10, 11). In the absence of T-cell recovery, this B-cell proliferation may prove fatal, as in the immunosuppressed inoculated hamster and in the kindred with B-cell rather than T-cell predominance in the peripheral blood.

HYPERTHYROIDISM

Like infectious mononucleosis, hyperthyroidism may be due to a marginally controlled proliferation of stimulated B lymphocytes prevented from assuming the clinical appearance of malignant lymphoma by suppressor T-cell activity. There is fragmentary evidence in support of this concept. Unexplained lymphocytic infiltration of soft tissue, including muscles and the thyroid itself, is now a well-recognized finding in some cases of hyperthyroidism (12). An immunoglobulin named long-acting thyroid stimulator (LATS) is found in the sera of approximately half the hyperthyroid patients examined (13), and this immunoglobulin was recently shown to be secreted *in vitro* by stimulated B lymphocytes of affected patients (14). The presence of other clinically deleterious autoimmune antibodies in the sera of hyperthyroid patients, reported independently by Anderson et al. (15), Doniach et al. (16), McNicol (17), and Blizzard et al. (18), seems to reflect poor control of immunoglobulin-synthesizing B cells. The increased incidence of malignant lymphoma in patients with long-standing hyperthyroidism, reported by Ulmann et al. (19), further attests to the relatively poor control of lymphocyte proliferation in these patients. Presumably, the increased number of T cells in hyperthyroid patients (20) prevents the evolution of malignant lymphoma from becoming the rule rather than the occasional exception.

DILANTIN-INDUCED LYMPHADENOPATHY

Finally, Kaplan (21) recently reviewed the literature documenting the occasional marked, but reversible, lymphadenopathy accompanying the administration of the hydantoin derivatives, especially Dilantin. The experimental conditions already cited for lymphoma development may be met in patients receiving Dilantin because it has been shown by Rausing and Trell (22) to stimulate cell proliferation in culture and by Sorrell et al. (23) to suppress T-cell activity or cell-mediated immunity in man. It is therefore not surprising that Charlton and Lundsford (24) found an association between Dilantin ingestion and lymphoma, which was 4 times the expected concordance.

CONCLUDING REMARKS

Although this discussion of suppressor T-cell activity has focused on the B cell as the target of this suppression, recent observations suggest the existence of T-cell regulation of T-cell reactivity as well. These observations include thymocyte inhibition of a graft-versus-host reaction (25)

and of responses to H-2 antigens (26). Lymphomas arising in an environment of combined antigenic stimulation and T-cell immunosuppression may therefore, at least theoretically, be of B-cell or T-cell origin.

A better understanding of the suppressor T-cell defect in these situations might allow for specific correction of the defect and reproducible induction of lymphoma regression analogous to the regressions regularly observed in infectious mononucleosis and Dilantin-induced lymphadenopathy.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966, pp 338-356
- (2) SALVADOR AH, HARRISON EG JR, KYLE RA: Lymphadenopathy due to infectious mononucleosis: Its confusion with malignant lymphoma. *Cancer* 27:1029-1040, 1971
- (3) JAFFÉ ES, SHEVACH EM, FRANK MM, et al: Nodular lymphoma—evidence for origin from follicular B lymphocytes. *N Engl J Med* 290:813-819, 1974
- (4) HENLE W, HENLE G: Epstein-Barr virus: The cause of infectious mononucleosis. In *Oncogenesis and Herpes Viruses* (Biggs PM, de Thé G, Payne LN, eds.). Lyon, International Agency for Research on Cancer, 1971
- (5) PATTENGALE PK, SMITH RW, GERBER P: Selective transformation of B lymphocytes by E.B. virus. *Lancet* 2:93-94, 1973
- (6) ADAMS RA, POTHIER L, HELLERSTEIN EE, et al: Malignant immunoblastoma: Immunoglobulin synthesis and the progression to leukemia in heterotransplanted acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphoma and infectious mononucleosis. *Cancer* 31:1397-1407, 1973
- (7) BAR RS, DELOR CJ, CLAUSEN KP, et al: Fatal infectious mononucleosis in a family. *N Engl J Med* 290:363-367, 1974
- (8) SHELDON PJ, HEMSTED EH, PAPAMICHAIL M, et al: Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet* 1:1153-1155, 1973
- (9) HAIDER S, COUTINHO M DE L, EMOND RT, et al: Tuberculin anergy and infectious mononucleosis. *Lancet* 2:74, 1973
- (10) WOLLHEIM FA, WILLIAMS RC JR: Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M (IgM) increase in infectious mononucleosis. *N Engl J Med* 274:61-67, 1966
- (11) SUTTON RN, REYNOLDS K, ALMOND JP, et al: Immunoglobulins and EB virus antibodies in infectious mononucleosis. *Clin Exp Immunol* 13:359-366, 1973
- (12) WERNER S: *The Thyroid* (2d ed). New York, Harper & Row, 1962, p 667
- (13) HAMILTON CR JR, MALOOF F: Unusual types of hyperthyroidism. *Medicine* 52:195-215, 1973
- (14) WALL JR, GOOD BF, FORBES IJ, et al: Demonstration of the production of the long acting thyroid stimulator (LATS) by peripheral lymphocytes cultured in vitro. *Clin Exp Immunol* 14:555-561, 1973
- (15) ANDERSON JR, GRAY KG, MIDDLETON DG, et al: Autoimmunity and thyrotoxicosis. *Br Med J* 2:1630-1632, 1964
- (16) DONIACH D, ROITT IM, TAYLOR RB: Autoimmune phenomena in pernicious anemia. Serologic overlap with thyroiditis, thyrotoxicosis and SLE. *Br Med J* 1:1374-1379, 1963
- (17) MCNICOL GP: Thyrotoxicosis associated with pernicious anemia. *Am J Med Sci* 241:336-342, 1961
- (18) BLIZZARD RM, CHANDLER RW, KYLE LM, et al: Adrenal antibodies in Addison's disease. *Lancet* 2:901-903, 1962
- (19) ULTMANN JE, HYMAN GA, CALDER B: The occurrence of lymphoma in patients with long-standing hyperthyroidism. *Blood* 21:282-297, 1963
- (20) AOKI N, WAKISAKA G, NAGATA I: Increase of T cells in Graves' disease. *Lancet* 2:49-50, 1973
- (21) KAPLAN H: *Hodgkin's Disease*. Cambridge, Mass., Harvard Univ Press, 1972, pp 31-36
- (22) RAUSING G, TRELL E: Malignant lymphogranulomatosis and anticonvulsant therapy. *Acta Med Scand* 189:131-136, 1971
- (23) SORRELL TC, FORBES IJ, BURNES FR, et al: Depression of immunological function in patients treated with phenytoin sodium. *Lancet* 2:1233-1235, 1971
- (24) CHARLTON MH, LUNDSFORD D: Le sostanze di idantoina come possibili cause del linfoma maligno. *Minerva Med* 62:15, 1971
- (25) GERSHON RK, COHEN P, HENCIN R, et al: Suppressor T cells. *J Immunol* 108:586-590, 1972
- (26) GERSHON RK, LIEBHABER SA: The response of T cells to histocompatibility-2 antigens. Dose response kinetics. *J Exp Med* 136:112-127, 1972

Spontaneous Regression in Choriocarcinoma and Related Gestational Trophoblastic Neoplasms¹

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SUMMARY—Gestational trophoblastic neoplasia represents a biologic spectrum of tumors progressing from the hydatid mole, to invasive mole, and to choriocarcinoma. This progression is reflected in increasing degrees of aneuploidy in the respective lesions. Just as there is a natural tendency for the rejection of the trophoblast of a normal pregnancy culminating either in parturition or in spontaneous abortion, rejection of tumors of trophoblast occurs at any point in the progression of the disease spectrum. The unusual effectiveness of chemotherapy in trophoblastic disease may be related to this natural tendency to rejection. This tendency, in turn, is thought to derive from the genetic disparity between the maternal host and the tumor tissue of fetal origin, since the fetus possesses both maternal and fetal antigens.—*Natl Cancer Inst Monogr* 44: 59–60, 1976.

We have applied the generic term “gestational trophoblastic neoplasia” to those lesions arising in pregnancy that fulfill the morphologic criteria of hydatidiform mole, invasive mole, and choriocarcinoma. In turn, these morphologic criteria are identified as those set forth by Novak and Seah (1). Thus a hydatidiform mole is a lesion derived from the chorionic tissue consisting grossly of a mass of villi that have become cystic and distended with a mucoid material. Histologically, the vasculature of these villi are impaired or obliterated and the stroma is highly edematous and sparse. The trophoblast has either an essentially normal appearance or varying degrees of excessive proliferative activity. However, there is no evidence of trophoblastic invasion beyond the normal level of endometrial attachment, the myometrium remaining entirely free of invasion. Clinically, no evidence of disease beyond the uterine cavity is usually demonstrable.

In contrast, an invasive mole (chorioadenoma destruens) is a lesion in which the trophoblast shows a high degree of proliferative activity leading to invasion of both the myometrium and the pelvic veins and, at times, even producing peripheral metastases to brain or lung. Histologically, residual villous structures persist irrespective of the extent and dissemination of the disease. It is this persistence of villi or villous remnants that is the distinguishing feature of the invasive mole.

Also in contrast, the lesion referred to as choriocarcinoma is made up entirely of trophoblast. It is characterized further by excessive vascularity with a tendency to hemorrhage, thrombosis, and necrosis. This lesion is highly invasive and metastasizes extensively.

In addition to these distinguishing histologic features, recent observations have indicated that among hydatid moles the trophoblast cells of almost all specimens exhibit a euploidic chromosomal pattern. However, an undetermined proportion of cases of invasive mole show distinct

patterns of aneuploidy. In contrast, all specimens of choriocarcinoma exhibit a high degree of aneuploidy, the chromosomal numbers ranging from 80 to 106 as compared with the normal human chromosome count of 46 (2, 3).

This progression in the degree of aneuploidy associated with increasing invasiveness and malignancy supports the concept that gestational trophoblastic neoplasia constitutes a biologically dynamic spectrum of disease. Such a view is also extensively supported by numerous features of the natural history of trophoblastic disease. For example, over 60% of patients found to have choriocarcinoma have a documented history of a prior hydatid mole. Our experience demonstrates the frequency of progression from this usually self-limited process to choriocarcinoma. In fact, about 25% of patients in whom the initial disease does not completely subside within 60 days following the primary evacuation of a hydatid mole (4, 5) will subsequently develop chorioadenoma destruens or choriocarcinoma. The clinical term applied under these circumstances is “persistent trophoblastic disease.”

This view does not preclude the possibility of a choriocarcinoma arising from trophoblast that has not previously been in the villous state. Such rare lesions fulfilling all of the morphologic criteria for choriocarcinoma have been termed “choriocarcinoma ab initio.” Probably, many of the choriocarcinomas first identified in the postpartum period also represent such an initially malignant process.

Nevertheless, the karyologic and nosologic evidence for progression from a less malignant phase of the disease to a more malignant phase indicates that in most instances the clinical course of events is best rationalized by such a concept.

Meanwhile, it is increasingly clear that host factors are important in determining the course of events in any given patient. As in the case of a normal pregnancy, there exists a continuing process of adjustment between the invading fetal tissue and the maternal host. In a normal pregnancy, rejection of the fetus may occur at any stage either before or after the fetus obtains viability. Similarly, in trophoblastic disease the process may be terminated by maternal rejection of the invading fetal tissue. In the case of a hydatid mole, such rejection may be complete or partial, and any retained trophoblast may be further rejected or may persist as an invasive mole or as a choriocarcinoma.

One of the clearest evidences of this host-tumor adaptation is the remarkable correlation between duration of disease and the frequency of complete remissions in response to chemotherapy (6). Such data suggest that in the management of trophoblastic disease, chemotherapy is simply serving as an adjunct to the continuing process of maternal rejection.

Hence the phenomenon of spontaneous regression must be carefully appraised not only on the customary grounds of histologic verification of diagnosis but with a full appreciation of the dynamic course of the trophoblastic disease process. To speak of spontaneous regression of either hydatid or invasive mole would simply be to describe the

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high frequency of tumor rejection in these instances. This would not be the equivalent of describing a spontaneous regression in such an inexorably malignant process as histologically proved bronchogenic carcinoma. However, in the case of histologically proved choriocarcinoma, spontaneous regression may be somewhat more clearly differentiated from the natural course of the disease. This phase of the disease, when not treated by specific chemotherapy, surgery, and supportive measures, clearly has a low incidence of recovery. Existing data do not permit a quantitative indication of the frequency of recovery in the absence of modern chemotherapeutic management.

Brewer et al. (7) provided an estimate of about 22% survival in women with histologically characteristic metastatic choriocarcinoma, who were managed by the classical methods of surgery, radiation, and general supportive measures. The extent to which this figure exceeds the frequency of completely spontaneous regression is purely speculative. However, documented instances of regression in such untreated cases have been recorded. Everson and Cole (8) have provided a tabulation of such cases. In addition, scattered reports abound in the literature, and it becomes clear that spontaneous regression occurs even with extensive metastatic disease. Hence this admittedly rare but quantitatively indeterminate event must be taken into account in the quantitative evaluation of a given therapeutic modality.

Moreover, the possible mechanism of the unusual effectiveness of chemotherapy in metastatic gestational trophoblastic disease as compared with other forms of metastatic cancer may indeed rest on this natural tendency for maternal rejection of both normal and malignant trophoblasts. Accordingly, extensive speculation has arisen as to the basis for this presumed impetus for rejection.

Classical immune mechanisms have been invoked and have provided the rationale for a number of immunotherapeutic efforts (9, 10). Also, efforts have been made to relate maternal lymphocyte response to the clinical course of trophoblastic disease. Because of the extraordinarily variable clinical course of this disease spectrum, each of these studies lacks sufficient numbers to provide any statistically valid basis for the varied findings developed.

Much of the current thinking about the immunologic aspects of this problem stems from the presumed analogy between graft rejection and maternal rejection of normal fetal trophoblast. Such an analogy fails to give due weight to the fact that the normal fetus develops its own vasculature and blood elements, whereas even an isograft derives its blood supply from the host. In the case of trophoblastic tumor tissue prior to maternal invasion, the vascular relationships are essentially equivalent to those encountered in a normal pregnancy. When the trophoblastic process extends beyond the uterine cavity, the vascular and blood elements are then derived from the host. Hence the mechanisms involved in the very ready rejection of intrauterine trophoblast may be expected to differ substantially from the relatively infrequent rejection of trophoblast invading the uterine wall or extrauterine sites.

In this connection, it is worth noting that metastatic choriocarcinoma is unique in the relative rarity with which the primary lesion is identifiable at autopsy (11). In most instances, the uterine cavity and endometrium are free of disease despite the active proliferation of metastatic lesions which kill the patient. However, intramural uterine lesions are among the most refractory lesions encountered in the uterus during chemotherapy (6). This

suggests some local intraluminal or endometrial factor favoring the demise of abnormal trophoblast.

It would seem that if classical immune mechanisms were operative in the rejection of malignant trophoblast, patients recovering after chemotherapy for extensive metastatic disease should prove intolerant to subsequent pregnancies, particularly when sired by the same father. Abundant clinical experience now shows that subsequent pregnancies occur in essentially normal frequency in such women and that the spontaneous abortion rate is only slightly increased, if at all. Hence if an immune response is a factor in the favorable response to chemotherapy, the effect must be a transient one.

It would seem more probable that the genetic disparity between the invading fetal tissue and the maternal host would favor either the spontaneous rejection of the tumor or would possibly augment the susceptibility of the tumor cells to chemical attack.

A more plausible basis for the remarkable responsiveness of malignant trophoblast to chemotherapy is the extraordinary vascularity of this type of tumor. Volkman (12) has described a tumor factor capable of inducing the proliferation of new vascular elements. Tumors of the trophoblast could be expected on morphologic grounds to be an abundant source of such a factor. In all events, ready access to a trophoblast tumor via the circulation would favor exposure to effective blood levels of medication.

One is obliged to conclude that as yet unidentified mechanisms probably unrelated to classical immune processes are operative in the apparently continuous struggle between invading trophoblast and maternal host for that tranquil symbiosis called a normal pregnancy, which, however, terminates in time by that gratifying form of rejection we call parturition. It is our good fortune that malignant trophoblast shares to a limited extent in this process and that modern chemotherapy synergizes so effectively with this inherent tendency to spontaneous regression.

REFERENCES

- (1) NOVAK E, SEAH CS: Choriocarcinoma of the uterus: Study of 74 cases from Mathieu registry. *Am J Obstet Gynecol* 67:933-954, 1954
- (2) HASEGAWA T: *Trophoblastic Neoplasia: Its Basic and Clinical Aspects*. Tokyo, Igaku Shoin, 1971, p 385
- (3) GALTON MP, GOLDMAN B, HOLT S: Karyotypic and morphologic characterization of a serially transplanted human choriocarcinoma. *J Natl Cancer Inst* 31:1019-1026, 1963
- (4) BREWER JI, GERBIE AB, DOLKART R, et al: Chemotherapy in trophoblastic diseases. *Am J Obstet Gynecol* 90:566-578, 1964
- (5) DELFS E: Quantitative chorionic gonadotropin: Prognostic value in hydatid mole and chorioepithelioma. *Obstet Gynecol* 9:1-24, 1957
- (6) HERTZ R, LEWIS J, LIPSETT MB: Five years experience with chemotherapy of metastatic choriocarcinoma and related trophoblastic tumors in women. *Am J Obstet Gynecol* 82:631-640, 1961
- (7) BREWER JI, SMITH RT, PRATT GB: Absolute 5 year survival rates of 122 patients treated by hysterectomy. *Am J Obstet Gynecol* 85:841-843, 1963
- (8) EVERSON TC, COLE WH: *Spontaneous Regression of Cancer*. Philadelphia, Saunders, 1966, p 280
- (9) BARDAWIL WA, TOY BL: The natural history of choriocarcinoma, problems of immunity and spontaneous regression. *Ann NY Acad Sci* 80:197-261, 1959
- (10) BAGSHAWE KD: *The Clinical Biology of the Trophoblast and Its Tumors*. London, Arnold, 1969, p 360
- (11) OBER WB, EDGECOMB JH, PRICE EB: The pathology of choriocarcinoma. *Ann NY Acad Sci* 172:299-321, 1971
- (12) VOLKMAN J: Tumor angiogenesis: Therapeutic implications. *N Engl J Med* 285:1182-1188, 1971

Spontaneous Remission in Burkitt's Lymphoma^{1,2}

John L. Ziegler, M.D.^{3,4}

SUMMARY—Three cases of spontaneous regression of Burkitt's lymphoma are reviewed and additional clinical and laboratory observations relevant to the role of host defenses are summarized. Spontaneous regression of Burkitt's lymphoma provides compelling evidence for antitumor immunity.—*Natl Cancer Inst Monogr* 44: 61-65, 1976.

Spontaneous regression of Burkitt's lymphoma has been well documented in the literature and provides compelling evidence for the existence of antitumor immunity in this disease (1-4). By definition, "spontaneous" regression is a misnomer in that a surgical biopsy must have been performed in order to establish the diagnosis, and hence there has been physical interference with the tumor. All patients with cases of spontaneous remission of Burkitt's lymphoma underwent such a procedure, and it would be extremely hazardous to accept documentation of such cases lacking histologic or cytologic confirmation in view of the many conditions that clinically mimic the disease (5).

The diagnosis of Burkitt's lymphoma rests on the characteristic histologic and cytologic morphology of the tumor cells. Histologically, the tumor is defined by the Rappoport classification as malignant lymphoma, undifferentiated, Burkitt's type (6). At low-power magnification, there is a "starry sky" appearance because of the many interspersed histiocytes that appear to be ingesting tumor debris. The tumor cells can best be characterized cytologically as undifferentiated lymphoblasts. Of particular note is the uniformity of cell size and the lack of nuclear maturation. Multiple nucleoli and finely reticular chromatin characterize the nucleus, and the cytoplasm is basophilic, containing abundant vacuoles.

CASE REPORTS

Three instances of clearly documented spontaneous complete regressions in Burkitt's lymphoma have been reported from Kampala, Uganda (1-4).

The first patient was a 36-year-old Muganda woman from Kyadondo district, living near Kampala. She presented in April 1966 with a complaint of bilateral swellings of the breasts and right shoulder of 1 month's duration (fig. 1). She had been breast feeding a 5-month-old baby. A biopsy taken at another hospital could not be traced, and a second biopsy revealed Burkitt's lymphoma. The first biopsy was

subsequently located and confirmed this diagnosis. Treatment was postponed because the patient requested to attend a funeral and promised to return in 3 days. She failed to return and, traced 6 weeks later, had no palpable evidence of tumor (fig. 2). The patient has been followed and remains in complete remission 8 years later. No adverse effects were noted in the breast-fed infant. This patient was reported originally by Burkitt and Kyalwazi (2) and has been followed subsequently by the Uganda Cancer Institute team in Kampala.

The second documented case of complete regression occurred in a 4-year-old girl who presented to the Mulago Hospital with a left maxillary tumor; biopsy revealed Burkitt's lymphoma (2). Treatment was delayed because of severe anemia. Blood transfusion was refused by the mother, however, and the child was removed from the hospital before cytotoxic therapy could be begun. She was traced 1 year later and had no evidence of tumor. Subsequent follow-up reveals that she has remained disease free.

The third patient was an 8-year-old boy who developed a swelling of the right orbit in June 1970 (4). A biopsy of the supraorbital mass was interpreted grossly as a "lipoma," but a histologic study was not performed. The child remained well for 6 months, at which time right orbital swelling recurred, and he was admitted to the ophthalmologic service at the Mulago Hospital where an infraorbital biopsy was performed on December 3, 1970 (fig. 3). During processing of the biopsy, the child developed a typical measles exanthem and a low-grade fever. On December 13, the tumor was noted to be regressing; the biopsy was read as Burkitt's lymphoma. The child was kept under observation, and by December 21, 18 days after the biopsy, there was complete clinical regression of the tumor (fig. 4). Serologic studies confirmed the diagnosis of measles by a typical rise and subsequent fall of complement-fixing antibody.

The child remained in complete remission until June 1971, when right orbital swelling recurred. Speculating that the measles infection may have had some connection with the apparent spontaneous regression, the child received vaccination with the Edmonson strain of measles vaccine, but tumor growth continued during the ensuing week of observation, despite an anamnestic antibody response. Complete regression was then achieved with the administration of cyclophosphamide.

Immunologic studies of this third patient revealed elevated antibody titers to the Epstein-Barr virus (EBV) capsid antigen, which did not fluctuate with the clinical course. Cutaneous anergy to recall skin tests and to dinitrochlorobenzene was documented at 1 and 3 months following spontaneous regression. His subsequent course has been characterized by continued recurrent tumors of the right orbit involving the sclera, conjunctiva, and periorbital tissues, with complete regressions achieved in each instance following cyclophosphamide. The child has remained in complete remission since his last orbital recurrence in July

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⁴ The data presented in this report are the result of collaborative studies involving many individuals. In particular, I wish to thank Dennis Burkitt, LeRoy Fass, Avrum Bluming, Richard Morrow, Ian Magrath, and Sebastian Kyalwazi, all of whom assisted in the care of the patients and whose observations are included in the text.

1972, and has shown no evidence of tumor deposits elsewhere in the body.

DISCUSSION

The genesis of spontaneous, complete remission in these patients is highly speculative. Clearly, one of the common denominators was that the patients had small, relatively localized tumors on which surgical biopsy (though not complete excision) was done. It is quite possible that such interference, both by reduction of tumor mass and by traumatic release of tumor antigen during the procedure, may have tipped the balance in favor of the host in accomplishing the removal of residual tumor through immunologic effector mechanisms. The role of hormonal factors in the first (a lactating patient with breast tumors) and the influence of measles infection in the third patient can only be speculative (4).

Other unique aspects of the clinical behavior of Burkitt's tumor and of certain correlative immunologic studies deserve comment because they provide further evidence for host defense mechanisms in this tumor. These clinical features, aside from those of the aforementioned spontaneous remissions, include: 1) occurrence of prolonged tumor regressions after low-dose or minimal chemotherapy (7); 2) observation of remissions after apparent nonspecific therapy with a compound called "septicemine" (hexamine iodomethylate, hexamine, sodium benzoate, and saccharose in distilled water), which is presumably inert with respect to cytotoxic activity (8); 3) occurrence of tumor regression after apparent failure of primary chemotherapy (3); 4) occasional patients whose tumors partially regress or remain stable for prolonged periods before treatment is instituted (3); 5) two documented instances of tumor regression after transfusion of convalescent plasma from patients who achieved durable remissions (1, 9); 6) primary anatomic location of tumor deposits in extranodal sites and lack of regional node involvement despite the obvious drainage of tumor cells into these nodal areas; 7) presence of abundant macrophages within the tumor tissue, which appear to be actively phagocytosing tumor debris; 8) in vitro suppression of cultured tumor growth by serum from patients in remission (3); 9) demonstration of delayed cutaneous hypersensitivity to autologous tumor extracts and the correlation of such reactivity with remission status and remission duration (10); and 10) demonstration of antibody responses to EBV-associated antigens in Burkitt tumor cells and the correlation of some of these antibody levels with the clinical behavior of the tumor (11).

Although it is beyond the scope of this presentation to discuss in detail the many investigations into the host defense mechanisms in Burkitt's lymphoma, it is instructive to conclude with some recent data relevant to the obvious implications of these observations, namely, immunotherapy.

Early studies in Kampala (10, 12) and Nairobi (13) led to the conclusion that some degree of impaired cell-mediated immunity, both nonspecific and tumor-specific, was associated with excessive tumor burden, an increased probability of relapse, and a poor prognosis. Speculating that BCG immunotherapy, because of its known potentiating effect

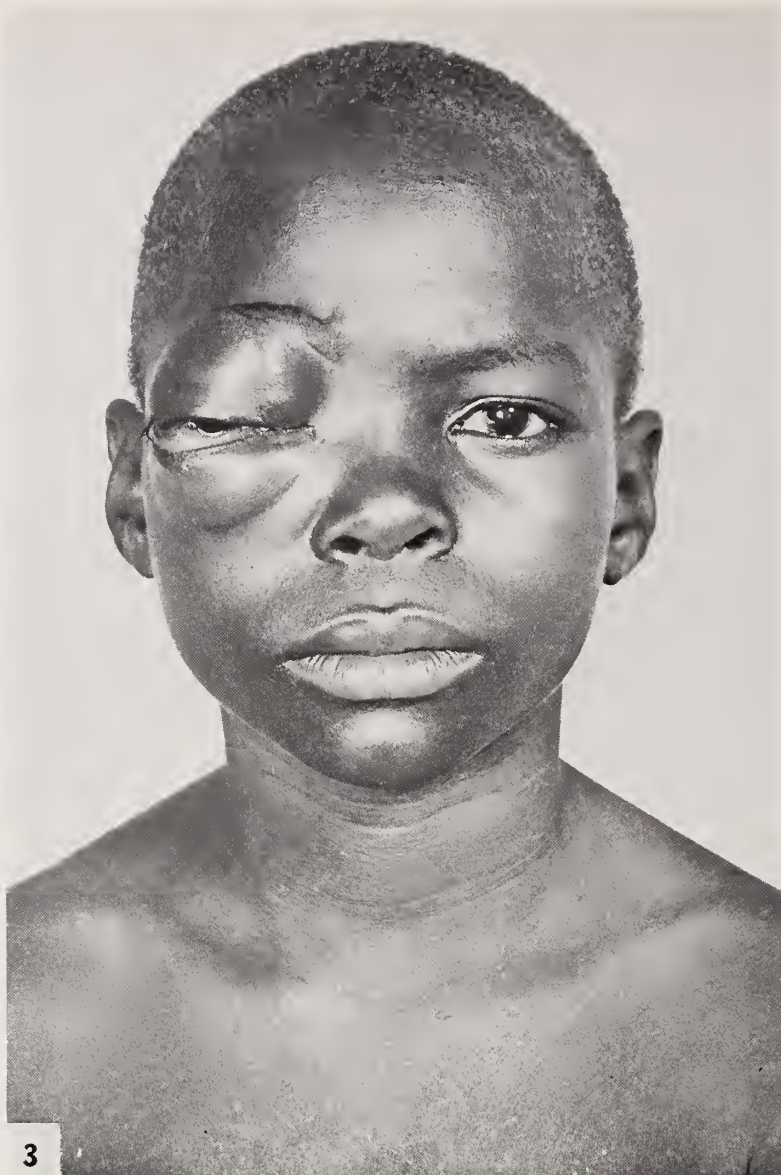
on cellular immunity (14), might reverse the anergy noted in these poor-risk patients, a study was launched in 1971 to examine the remission-prolonging effect of BCG in patients with remissions induced by cyclophosphamide. This study confirmed that a large tumor burden was reflected by cutaneous anergy to standard recall skin test antigens and that reactivity to these antigens returned when remission status was achieved. BCG immunotherapy administered early in remission, with Pasteur Institute vaccine given by scarification over a period of 10 weeks, significantly accelerated the recovery of cutaneous hypersensitivity but failed to prevent relapse, as compared with concomitantly randomized as well as matched historical control patients who received no immunotherapy (15). In this study, cutaneous hypersensitivity appeared to be of secondary importance in tumor-associated immunity in Burkitt's lymphoma, and further studies should perhaps be directed toward the role of humoral antibodies in the immunologic control of the tumor. In conclusion, there is compelling evidence for tumor immunity in Burkitt's lymphoma, but the biologic aspects of the host-tumor relationship remain obscure.

REFERENCES

- (1) BURKITT DP: Clinical evidence suggesting the development of an immunological response against African lymphoma. In *Treatment of Burkitt's Tumor* (Burchenal JH, Burkitt DP, eds.). New York, Springer-Verlag, 1967, pp 196-208
- (2) BURKITT DP, KYALWAZI SK: Spontaneous remission of African lymphoma. *Br J Surg* 21:14-16, 1967
- (3) NGU VA, BURKITT DP, OSUNKOYA BO: Clinical and related evidence of host defence mechanisms. In *Burkitt's Lymphoma* (Burkitt DP, Wright DH, eds.). London, Livingstone, 1970, pp 158-163
- (4) BLUMING AZ, ZIEGLER JL: Regression of Burkitt's lymphoma in association with measles infection. *Lancet* 1:105-106, 1971
- (5) ZIEGLER JL, WRIGHT DH, KYALWAZI SK: Differential diagnosis of Burkitt's tumor of the face and jaws. *Cancer* 27:503-514, 1971
- (6) BERARD CW, O'CONOR GT, THOMAS LB, et al: Histological definition of Burkitt's tumor. *Bull WHO* 40:601-607, 1969
- (7) BURKITT DP: Long-term remissions following one- and two-dose chemotherapy for African lymphoma. *Cancer* 20:256-259, 1967
- (8) DAVID J, BURKITT DP: Burkitt's lymphoma: Remissions following seemingly non-specific therapy. *Br Med J* 4:288-291, 1968
- (9) NGU VA: Host defences to Burkitt tumour. *Br Med J* 1:345-347, 1967
- (10) FASS L, HERBERMAN RB, ZIEGLER JL: Delayed cutaneous hypersensitivity reactions to autologous extracts of Burkitt-lymphoma cells. *N Engl J Med* 282:776-780, 1970
- (11) GUNVEN P, KLEIN G, HENLE W, et al: Immunological studies on a case of recurrent Burkitt's lymphoma during immune stimulant treatment. *Int J Cancer* 12:115-123, 1973
- (12) ZIEGLER JL, BLUMING AZ, FASS L, et al: Relapse patterns in Burkitt's lymphoma. *Cancer Res* 32:1267-1272, 1972
- (13) STJERNSWÄRD J, CLIFFORD P, SVEDMYR E: General and tumour-distinctive cellular immunological reactivity. In *Burkitt's Lymphoma* (Burkitt DP, Wright DH, eds.). London, Livingstone, 1970, pp 164-171
- (14) BLUMING AZ, VOGEL CL, ZIEGLER JL, et al: Immunological effects of BCG in patients with malignant melanoma. A comparison of two modes of administration. *Ann Intern Med* 76:405-412, 1971
- (15) McGRATH IT, ZIEGLER JL: Failure of BCG immunostimulation to affect the clinical course of Burkitt's lymphoma. *Br Med J* 1:615-618, 1976



FIGURES 1, 2.—A 36-year-old woman developed bilateral breast masses while nursing her 5-month-old baby. A biopsy revealed Burkitt's lymphoma. The patient left hospital before treatment, and when traced 6 weeks later, her disease was found to be in complete regression.





FIGURES 3, 4.—An 8-year-old Ugandan boy developed a right orbital swelling, a biopsy of which revealed Burkitt's lymphoma. Treatment was delayed because the child developed measles following the biopsy. Eighteen days after the biopsy, the tumor had completely regressed. *Note* the exfoliative measles exanthem.

Spontaneous Regression of Malignant Melanoma: A Review of the Literature on Incidence, Clinical Features, and Possible Mechanisms^{1,2}

Larry Nathanson, M.D., F.A.C.P.³

SUMMARY—A review of the clinical features of spontaneous regression of malignant melanoma was undertaken. Thirty-three patients with total regression of primary melanoma ("primary regressors") and 40 (13 of whom were somewhat doubtful) with regression of metastatic disease were reviewed in detail. These patients appeared to represent a typical age incidence of melanoma but the primary regressors showed an unexpected predominance of male over female patients. A variety of unique clinical features of the histories of the patients were noted, but none appeared to explain the regression with any degree of predictability. Cutaneous metastases constituted the most common site of regression, followed, in order, by lymphatic, pulmonary, and hepatic metastases. About 40% of patients with spontaneous regressions appeared to have "spontaneous cure," which implies that the disease had not relapsed either during a long period of follow-up or until death from some other cause. Mechanisms that possibly relate to spontaneous regression of melanoma fall into the following general categories: immunologic, endocrine, pigment metabolic, intracellular, nutritional, and carcinogenic. Further quantitative studies of patients actually undergoing spontaneous regression or the development of a model of spontaneous regression may be a key to our understanding of this interesting "experiment of nature."—*Natl Cancer Inst Monogr* 44: 67-76, 1976.

A significant body of evidence suggests that factors initiating regression of at least some aggregates of tumor cells in human malignant melanoma are a frequent event in the natural history of the disease. The effectiveness of these factors ranges from regression and depigmentary changes in clusters of cells in primary lesions to total regression in advanced metastatic disease. The purposes of this paper are to document the above-mentioned hypothesis and to emphasize the fact that regression of metastatic melanoma is a true, albeit rare, event.

Spontaneous regression of malignant melanoma must include at least the following criteria (1): 1) a clinical and histologic diagnosis of melanoma; 2) lack of any therapeutic manipulation that could account for regression; 3) clinical observation of regression of existing lesions, preferably documented with photographs, X-rays, and the like; 4) follow-up of patients until their death, or at least for many years; and 5) if possible, histopathologic studies of tissue sites where regression has presumably taken place. Using these general criteria, several authors have reviewed spontaneous regression of cancer (2-5), in addition to the classic monograph of Everson and Cole (1).

Clearly, there are problems encountered in the fulfillment of these criteria. First, the requirement of biopsy

proof of malignancy, in itself, implies a manipulation of the disease which, at least theoretically, could have some therapeutic effects. Second, increasingly aggressive treatment of cancer patients has resulted in the real possibility of a masking of any spontaneous regression that might have taken place. If spontaneous regression were to take place in a treated patient, one would invariably assume that the regression was related to the treatment.

Evidence in four areas supports the variety of reports of spontaneous regression in melanoma. These include 1) direct clinical observation of spontaneous regression, 2) characteristic histopathologic changes in regressing lesions, 3) an immunotherapeutic model in humans of regression of melanoma, and 4) experimental data on possible mechanisms that could explain the phenomenon of spontaneous regression in melanoma.

CLINICAL EVIDENCE OF HOST-DEFENSE FACTORS IN MELANOMA

Numerous clinical features of melanoma suggest the possibility that host-defense factors are significant in this disease.

Regressive Changes in Primary Melanomas

It has been emphasized by Mihm et al. (6) and others (7-9) (table 1) that focal areas of spontaneous regression, identifiable as white, gray-white, or pink-white areas, are hallmarks of primary melanoma, most commonly of the superficial, spreading variety. These areas correspond to areas of disappearance of pigmented cells, both benign and malignant. Although the regression in such lesions is delimited and occurs during progression of other parts of the primary lesion, nonetheless, it emphasizes the relatively common presence of underlying factors, visible expression of which could span the spectrum from slight to major changes in the natural history of the disease. In the case of primary melanoma of the uveal tract, mostly choroidal melanoma, spontaneous regression may be a common phenomenon. Reese et al. (10) identified three types of lesions: "cluster," "lytic," and "infarctive," which together may account for as many as 29% of choroidal lesions. He reported on 35 patients of whom 3 appeared to fulfill all the criteria of spontaneous regression of malignant choroidal melanoma. One patient also had a primary melanoma of the skin.

The Halo Nevus

A significant amount of study has delineated an important series of immunologic responses in patients with halo nevi (11, 12). These studies have suggested that both cellular immunity and humoral immunity toward melanoma-specific or associated antigens are present in patients with halo nevi. Epstein also reported the dramatic appearance of multiple halo nevi in association with removal of a primary malignant melanoma.

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Unknown Primary Melanoma

Many authors (table 2) have documented the incidence of unknown primary lesions in patients who present with metastatic melanoma. Of this group of 5.4% of all malignant melanomas, some patients could be accounted for on the basis of primaries in occult sites. However, some studies, the most significant of which is that of Smith and Stehlin (17) have produced strong evidence to suggest that these unknown primaries actually constitute spontaneous regression of primary melanoma, even during development of metastatic lesions.

Smith proposed the following criteria to present as evidence in favor of spontaneous regressions of unknown primary lesions: 1) a clinical history of a pigmented lesion (situated in an area drained by tumor-involved lymph nodes) the initial appearance of which was compatible with primary melanoma; 2) the absence of any other primary lesion identifiable on history or physical examination that could represent the original lesion; 3) the presence of a typical pigmented or depigmented change in the skin at the site of the untreated possible primary lesion; histologic changes in the excised site of such a possible primary lesion, which support the clinical evidence of regression of a primary malignant melanoma, such as an arrangement of melanophages around superficial capillaries, a sharp decrease in pigment in the area of the lesion, perivascular lymphocyte infiltration, degenerated atypical melanocytes or melanoma cells, and infiltration of polymorphonuclear leukocytes and/or eosinophils; and 4) the absence, histologically, of a configuration of primary malignant melanoma with or without the presence of melanoma cells in atypical distribution in the dermis or subcutaneous tissue at the site of the lesion. Table 3 presents 33 patients who appear to conform in large part to these criteria. Patient 33 is a previously unreported patient whose clinical history is included as an appendix and for whom photographic

TABLE 3.—*Spontaneous regression of malignant melanoma: Primary only*

Case No.	Reference	First author	Additional references, comment
1	(21)	Iijima	Melanoma with xeroderma (cited by Ronchese, 22)
2, 3	(14)	Pack	Long survivors
4, 5	(16)	Das Gupta	
6-14	(17)	Smith	
15	(23)	Todd	
16-24	(24)	Milton	9/12 cases fulfill criteria
25	(25)	Parache	Choroid primaries
26-28	(10)	Reese	3/22 cases fulfill criteria
29	(26)	Farnsworth	2 primary melanomata (metastasis to oligodendroglioma)
30	(27)	Brincker	
31, 32	(28)	Ikonopisov	
33		Nathanson	Not previously reported (see "Addendum")

documentation of the appearance and histopathology of the regressing primary is presented.

The phenomenon of common late recurrence of melanoma, in which 5-25 years may elapse between the time the primary is removed and the first recurrence is noted, has been well documented (19), and it is additional evidence of possible protective effects of host response to tumor. The factors that account for a long "free interval" clearly could relate either to the intrinsic generation time of the residual tumor cells (cell kinetic factors) or to host resistance. A number of observations have been made relating to in vivo generation time in melanoma (29). They suggest a highly variable generation time with a median of 3 days, together with a growth fraction of 20-30% of melanoma cells. The potential tumor doubling time as calculated from the generation time and growth fraction was shorter than the actual doubling time, which indicates that cell loss was in the range of 70% of the rate of cell production. The variability of such data leaves much to be desired, and other studies of volume doubling time (19) have estimated a 2-16-week volume doubling time with a mean of 7 weeks. Although calculations of cell kinetics based on such data involve many assumptions, including linear generation time when plotted against time or location of cells from the center to the periphery of the lesion, one can make a rough guess that a maximum of 6-9 years could be accounted for in terms of the simple time necessary for a single residual melanoma cell to multiply to the size of a clinically detectable lesion. Thus there is strong evidence to suggest that host factors may be responsible in a significant part for free intervals in excess of those mentioned above.

It is of further interest that the duration of "free interval" does not seem to be correlated with time from first recurrence to death [(30); Nathanson L: Unpublished data from the Eastern Cooperative Oncology Group studies]. This suggests the possibility that the biologic character of recurrent disease (a new "clone" of cells?) is different from that of the residual disease, as there is no obvious reason to suspect changes in host-mediated defense mechanisms. The corollary is also true (30). Following surgical treatment of primary tumor, detection of circulating tumor cells does not appear to result in a high rate of recurrence. This cir-

TABLE 1.—*Incidence of partial regression of primary melanoma*

Reference	First author	Incidence	Total No.	Percent
(6)	Mihm	—	—	—
(7)	Little	56	361	15.5
(8, 9)	McGovern	28	202	13.8
Total		84	563	14.9

TABLE 2.—*Malignant melanoma: Incidence of unknown primary lesions*

Reference	First author	Incidence	Total No.	Percent
(13) ^a	Lane	22	554	4.0
(14)	Pack	29	1,190	2.4
(15)	Charalambidis	8	250	3.2
(16)	Das Gupta	37	992	3.7
(17)	Smith	40	461	8.7
(18)	McGovern	83	613	7.0
(19)	Nathanson	6	143	4.2
(20)	Nathanson	10	141	7.1
Total		235	4,344	5.4

^a Including series of Catlin.

cumstance suggests a preponderant importance of host-defense factors in tumor suppression.

In particular, survival curves in melanoma (19) have shown that the decrement in survivorship in patients with this disease is linear for over 10 years, even when based on the survival of patients who eventually developed metastatic disease. Thus the decrement in survivorship in patients who have survived free of melanoma for 5 years, due to recurrence and death from the disease, occurs at the same rate between 5 and 10 years as 0 to 5 years.

Documented Spontaneous Regression of Metastatic Melanoma

A number of authors have attempted to estimate the incidence of spontaneous regression of metastatic malignant melanoma (table 4). Although these estimates are made with an unreliable numerator and denominator, nonetheless, when 8 such series are grouped together, comprising a total of almost 5,000 patients with melanoma, there is an average incidence of approximately 2.2 patients per 1,000. An attempt was made to review the world's literature on well-documented patients with regression of metastatic malignant melanoma (table 5). Such reports had to fulfill the above-mentioned criteria for the definition of spontaneous regression in order to be included. The first 19 patients listed in this table are identical to those reported by Everson and Cole (1) in 1966. In addition, 8 patients, who have been reported since that time, were added. It is clear from table 6, which presents reported spontaneous regressions of metastatic melanoma that failed to have adequate documentation, that this group of 27 patients is far from complete. Indirect references to spontaneous regressions that have not been reported are found in the literature (57, 60), and I know of additional word-of-mouth reports.

The clinical features of these patients are presented in table 7. The age range of patients spans that of the decades of highest incidence of melanoma. No significant difference was noted in median age among the patients who exhibited regression of metastatic or primary disease. There was a predominance of male over female patients with primary lesions regressing, but in the metastatic disease category the sexes were essentially equal in number. This is of interest in view of the fact that overall survival in females is significantly better than in males with this disease. Primary therapy of the patients with regressing metastases was excision in most cases, although cauterization of the primary occurred in 3 patients. Four patients of the 40 with regression of metastatic disease had primaries in the eye,

TABLE 4.—*Metastatic malignant melanoma: Incidence of spontaneous regression*

Reference	First author	Incidence	Total No.	Percent
(31)	Daland	1	174	0.58
(32)	Vogler	1	253	0.40
(14)	Pack	1	1,190	0.08
(33)	Cade	1	226	0.44
(34)	Block	1	236	0.42
(35)	Peterson	2	621	0.32
(30)	Moore	2	1,700	0.12
(20)	Nathanson	1	141	0.71
Total		10	4,541	0.22

representing 10% incidence, slightly higher than the usual incidence of such melanoma lesions, which is approximately 8%. In 24 of 33 patients with primary regressions, the site of the regressing lesion was subsequently excised, and histopathologic evidence of the regressing primary was reported.

Among the clinical clues that might be related to regression in the 27 patients reported in the metastatic disease group (table 5), the following are worthy of note: For 3 patients, blood transfusions had been administered sometime prior to the regression; in 1, antirabies inoculation for dog bites had been given prior to regression; and in 1, "hot air" baths preceded regression. Three regressions occurred after termination of pregnancy. For 23 patients, removal of the primary had occurred at some time prior to regression, but for 6 patients, it occurred relatively shortly before the onset of regression. For 1 patient, excision of an anterior abdominal wall lesion was followed shortly by regression. One patient had irradiation to metastases other than those that regressed, and 1 patient had irradiation to subcutaneous metastases that were observed to undergo no evidence of postradiation improve-

TABLE 5.—*Spontaneous regression of metastatic malignant melanoma*

Case No.	Reference	First author	Additional references, comments ^b
1	(36)	Mathews	
2	(31)	Daland	(37)
3	(38)	Pack	Antirabies vaccine; (39)
4	(40)	Levi	
5	(41)	Sumner	Unknown 1°; regressed at term of pregnancy; (42)
6	(43)	Allen	Regressed following term of pregnancy
7	(1)	Meyer ^a	Abcess near lesion
8	(1)	Vial ^a	¹³¹ I-labeled antibodies; (44)
9	(45)	Levison	1° choroid eye
10	(46)	Malleson	1° ?choroid eye
11	(47)	Boyd	Regressed following term of pregnancy
12	(47)	Boyd	
13	(32)	Vogler	Some radiation
14	(33)	Cade	
15	(1)	Ellis ^a	
16	(35)	Peterson	
17	(35)	Peterson	1° regressed+eutaneous metastasis
18	(1)	Baker ^a	Subcutaneous metastasis, spontaneous
19	(1)	McCredie ^a	Hepatic metastasis/transfusion
20	(22)	Ronchese	Xeroderma; ?multiple 1°
21	(48)	Stidolph	
22	(44)	Foley	
23	(30)	Moore	Garlie consumption
24	(49)	The	
25	(27)	Brincker	
26	(50)	Doyle	
27		Nathanson	Not previously reported (see "Addendum")

^a Cited by Everson and Cole (1) as personal communication.

^b 1°=primary cancer.

TABLE 6.—*Spontaneous regression malignant melanoma: Questionable cases*

Case No.	Reference	First author	Comments
1	(51)	Gould	Cited by Handley but not documented
2	(52)	Swan	Cited by Handley but not documented
3	(53)	Byrd	Not documented
4	(54)	Turner	Radiotherapy
5	(55)	Beswiek	Radiotherapy
6		Eastcott	Cited by Stildolph (48) but not documented
7	(42)	Sumner	Treated with transfusion
8	(56)	Eilber	See Eilber's Table 1; not documented
9	(57)	Berg	Not documented; cited by Bowden (58)
10	(30)	Moore	One of two cases not documented
11	(59)	Scheffer	Eye primary—not documented
12	(59)	Seheffer	Eye primary—not documented
13	(60)	Falk	Not documented

ment. One patient had an abscess near a mass of tumor that regressed. One patient stated that he had consumed garlic prior to the time of each of a series of regressions and that progression of the disease, which eventually resulted in death, was due to his inability to obtain garlic to eat. One patient had multiple primary melanomas and accompanying xeroderma pigmentosum. One patient was treated with ¹³¹I-labeled antibodies shortly before his regression. At least 1 patient had an unknown primary that may have possibly regressed prior to the regression of metastatic disease.

These factors, which include the potential for immunologic, endocrine, posttraumatic, infectious, nutritional, and genetic influences, are so heterogeneous that it is impossible to delineate any common theme.

The predominant regression in these patients was the disappearance of cutaneous metastases (15 patients). Six of these occurred with marked depigmentary effects, suggestive of the phenomenon of both halo nevus and of partial or complete regression of primary melanoma (19 of 31 patients exhibited this; see "Appendix"). Regression of metastatic deposits in lymph nodes, lung, and hepatic and cerebral sites was noted in occasional patients. Clearly such regression could have occurred permanently or temporarily. In the group reviewed, approximately half had a regression that lasted less than, and half greater than, 5 years. At the time of report with follow-up over 5 years, 14 of 35 patients for whom information was available were free of disease or had died from causes other than melanoma. Thus in approximately 40% of spontaneous regressions of metastatic disease, regression appeared to be permanent and could constitute "spontaneous cure."

POSSIBLE MECHANISMS OF SPONTANEOUS REGRESSION OF MALIGNANT MELANOMA

It is impossible to review in detail all studies of melanoma that might bear on the mechanisms of spontaneous regres-

TABLE 7.—*Spontaneous regression of malignant melanoma: Summary of clinical features*

Features	Regression of metastatic disease	Regression of primary only	Doubtful cases
Number	27	33	13
Age			
Range	20-74	27-57	22-70
Median	45	39	46
Sex			
Male	12	22	5
Female	14	8	3
Unknown	1	3	5
Primary therapy			
Excised	20		5
Cauterized	3		2
Unknown 1°	1	30 ^a	2
Eye primary	2	3	4
Nature of regression			
Cutaneous metastases	14		4
1°+ cutaneous metastases (with depigmentation)	1		
	6 ^b	33	4
Lung metastases	6	19	2
Pulmonary metastases + cutaneous	3		2
Hepatic + cutaneous	3		1
Hepatic + lung + cutaneous	1		1
?Brain	1		1
Unknown			4
Survival			
Death from melanoma	17	15	4
Alive or death from other disease	10	15	4
Unknown		3	5

^a 24 with excision of primary site.

^b Cases 5, 14, 15, 18, 20, 23 of table 5.

sion. Table 8 outlines some of the evidence, categorized in six general areas. Perhaps the most interesting and the best studied is the area of immunologic factors involved with host response to melanoma. However, endocrine, pigment metabolic, and nutritional factors, as well as other less studied areas, deserve comment. The brief comments below should be supplemented by examination of the literature cited in table 8.

Immunologic Factors

Histopathologic studies demonstrating infiltration of mononuclear cells, and other light microscopic evidence suggestive of immune reactions during spontaneous regression of primary melanoma have already been discussed. The association of halo nevi with occasional reports of vitiligo in regressing melanoma also suggests the possibility of autoimmune factors, presumably directed against cells that possess the metabolic apparatus necessary for pigment formation. In addition, dermatomyositis has been reported in association with malignant melanoma (68). The response of melanoma to vital vaccines, such as that

TABLE 8.—*Possible mechanisms of spontaneous regression of melanoma*

Factor	Reference	First author
Immunologic		
Pathologic data—light microscope-----	(17, 61, 25, 9, 62, 28)	Smith, Little, Parache, McGovern, Lloyd, Ikonopisov
Halo nevi-----	(63, 12, 64, 65)	Epstein, Lewis, Nicholls, Wayte
Autoimmunity and vitiligo-----	(66, 67)	Lerner, Bor
Associated dermatomyositis-----	(68)	Katz
Skin tests with tumor antigens-----	(69, 70)	Oren, Bluming
In vitro data		
Humoral immunity-----	(71-74)	The, Hellström KE, Hellström I, Morton
Cellular immunity-----	(75-82, 19, 20)	Clark, Mukherji, Nathanson
Rosette formation-----	(83)	Wybran
Response to viral, bacterial vaccines-----	(38, 19, 84-86)	Pack, Nathanson, Burdick, Milton, Hunter-Craig
Sympathetic ophthalmia-----	(87)	Easom
Endocrine		
Rare before puberty-----	(19)	Nathanson
Better female survival-----	(88-90)	Mackie, White, Stephenson
Better female survival premenopause-----	(19)	Nathanson
Peak incidence after menopause-----	(91, 92)	Sadoff, White
Improvement with termination of pregnancy-----	(91)	Sadoff
Worsening at menopause associated with melanocyte-stimulating hormone (MSH)-----	(93)	Klaus
ACTH-----	(94)	Orth
Human chorionic gonadotropin (HCG)-----	(95)	Braunstein
Hormone therapy-----	(96, 97)	Flax, Johnson
Ablative endocrine treatment-----	(98-101)	Wigby, Howes, Pack, Shimkin
Pigment products		
Tyrosinase inhibition-----	(102)	Chian
Toxicity of phenol melanogens-----	(103, 104)	Frenk, Hochstein
Relation to vitiligo-----	(105, 106, 66)	McGregor, Burdick, Lerner
Improvement with tyrosine-----	(107)	Demopoulos
Phenylalanine-deficient diet-----	(108)	Demopoulos
Penicillamine methyl tyrosine-----	(109)	Voorhees
Intracellular (nonpigmentary)		
Lysosomes-----	(110)	Mishima
Chalones-----	(111)	Bullough
Inhibitory factor-----	(112)	Seiji
Interference with nutrition		
Tumor angiogenic factor-----	(113)	Folkman
Characteristic, central necrosis, hemorrhage-----	(114, 10)	Brem, Reese
Cytokinetic-----	(29)	Shirakawa
Removal of carcinogens		
Actinic radiation-----	(115, 116)	Epstein, Lee
Trauma-----	(117)	Ghadially
Genetic factors-----	(118, 119)	Anderson, Wallace
Virus-----	(11)	Epstein
Chemical-----	(120)	Kirsch

to rabies vaccine, reported by Pack (38) as well as recent reports of immunotherapeutic responses to BCG vaccination (74, 81) also suggests the possibility that immunologic adjuvants may augment host immune response to the point at which clinical regression of disease is observed (121). Whether these immunotherapeutic models mimic the true mechanism of spontaneous regression is, at this time, unclear. Detailed studies of host immune response in melanoma have included in vivo studies of skin testing with tumor antigens (69, 70) and in vitro studies, including those of humoral immunity by Morton et al. (74) and of cellular immunity by many authors including the Hell-

ströms (73), and Clark, Nathanson, and Mukherji from 1971 to 1973 (19, 20, 75-82).

Wybran and colleagues (83) reported that metastatic melanoma patients possess a significantly decreased number of rosette-forming lymphocytes (presumably T cells). Diminution in number of rosette-forming cells was directly related to survival.

Detailed immunologic studies of patients actually undergoing spontaneous regression have been few, as might be expected by the rarity of the phenomenon and the only recently developed quantitative techniques to study it. The et al. (49) did tumor cell imprint preparations in a

regressing lesion, which revealed numerous large phagocytes in juxtaposition to melanoma cells. They were able to grow few of these cells in culture and demonstrated by immunofluorescence that the cells were coated with human immunoglobulins. These immunoglobulins were eluted from the tumor cells and observed to stimulate phagocytosis of melanoma cells in vitro to a degree not noted in appropriate phosphate-buffered saline controls.

As noted above, Epstein et al. (63) also reported a series of patients in whom removal of a primary melanoma was associated with the appearance of multiple prominent halo nevi. Lymphocytes were observed around typical degenerating melanoma cells.

Endocrine Factors

The association of spontaneous regression with termination of pregnancy, noted in 3 patients in the series, suggests the possibility of sensitivity of melanoma cells, at least at some time during their natural history, to endocrine factors. Another clinical observation suggesting an endocrine role is the rarity of melanoma prior to puberty; a detailed review of the world literature has revealed approximately 53 patients in whom adequate evidence documenting prepubertal melanoma is available. In addition, the superior survival rate of female patients, particularly in the premenopausal age group, a peak of incidence of melanoma just after menopause, and the possible association of growth of melanoma cells at least in vitro with MSH (93) or their production of various hormonal polypeptides including ACTH (94) and HCG (95) are also suggestive evidence. Sadoff et al. (91) emphasized the reports of activation of melanoma following estrogen administration. There are rare responses of human melanoma to antiestrogenic hormones, and endocrine sensitivity of pigmented animal tumors has been demonstrated. Such a progestational and antiestrogenic compound is 6- α -methyl-pregn-4-ene-3,11,20-trione (97).

Pigment Products

Studies by Frenk and Ott (103) and by Hochstein and Cohen (104) have suggested that certain phenol melanogens may have direct cytotoxicity for melanoma cells in vitro or in vivo. Lerner (66) has speculated that melanocyte control may be achieved by some mechanism that increases synthesis of such toxic phenol melanogens, resulting in autolysis of melanoma cells. Klaus and Snell (93) reported that hormones, specifically MSH, may well be used as a means for augmenting production of such phenol metabolites. Whether such a mechanism might account for spontaneous regression is speculative.

Inhibition of the tyrosinase pathway, by use of tyrosine- and phenylalanine-deficient diets (107), tyrosinase inhibitors such as penicillamine (108), or competitive inhibitors of tyrosinase such as α -methyltyrosine (109) has also been reported to inhibit growth of melanoma cells. Whether any of these techniques may be successful in humans in vivo or may account for spontaneous regression is debatable.

Intracellular Factors

Intracellular factors have been reported to have an inhibitory effect on melanoma cells. Mishima (110) suggested that lysosomes may have a primary role in autodegradation of melanoma cells and that hormonal or chemical enhancement of such lysosomal activity may

result in increased death of melanoma cells. In addition, Bullough and Laurence (111) studied the effect of epidermal "chalone" mechanism in melanoma. Preliminary data suggested that subcutaneous injections of pigskin chalones into NMRI mice bearing Harding-Passey melanomas resulted in regression of these tumors. Additional evidence in three other animal systems was reported by the same author, but to my knowledge, no corroborative data to support these studies have been reported.

An aqueous extract of Harding-Passey melanoma precipitated in alcohol has been demonstrated by Seiji et al. (112) to have a significant inhibitory effect on DNA and protein synthesis in malignant melanocytes in tissue culture.

Nutritional Factors

Factors that interfere with tumor cell nutrition, usually by restriction or interference with blood supply, may have a function in autodestruction of malignant tumors. Of great interest is the recent demonstration by Folkman and colleagues (113, 114) that tumors may produce an "angiogenic factor" responsible for the vascular endothelial proliferative effects necessary to establish adequate blood supply. Interference with the ability of a tumor to produce such a factor could reduce tumor blood supply, rendering tumor deposits nonviable. It has been emphasized by Shirakawa et al. (29), in cytokinetic studies of melanoma deposits, that growth rates of melanoma cells vary inversely with the distance of such cells from the microvasculature providing the blood supply for them. The rate of volume growth of a tumor deposit depends on the rate of generation of new cells less the rate of cell death. Thus quantitative estimates of the effects of decrease in nutrition depend upon careful studies of generation time and the size of the proliferative pool in such tumor cell deposits, and the effects cannot be judged simply on the volume doubling time. Nutritional factors in regression of melanoma are probably most clearly demonstrated in choroidal melanoma (10). The spontaneous infarctive or coagulative pattern of necrosis seen in this lesion strongly suggests a mechanism of ischemia resulting from interruption or inadequacy of blood supply.

Removal of Carcinogens; Genetic Factors

Early in tumor growth, the continued existence of the tumor may depend in part upon the continuing presence of carcinogenic factors, such as viral agents (11), actinic radiation (115), genetic factors (118), trauma (117), or chemical carcinogens (120). Epidemiologic and laboratory studies have suggested the association of actinic radiation with the genesis of melanoma, but one would assume that once such a malignant transformation had taken place, it would be a unidirectional and irreversible cellular change. Factors that relate melanoma to hereditary disease include: reports of familial melanoma; the high incidence of melanoma in fair-skinned individuals with Celtic surnames; the occasional association with neurofibromatosis (and cafe-au-lait spots in that disease); the association of benign freckling in Peutz-Jeghers syndrome; the increased incidence of melanoma in xeroderma pigmentosum, an hereditary disease; and the occasional association of melanoma with hereditary albinism. Whether any of these factors is related to spontaneous regression of melanoma is highly speculative, and to my knowledge, spontaneous regression of melanoma has never been reported in patients with the hereditary variety

of melanoma. However, in 2 patients reviewed in this group (1 with regression of a primary and 1 with regression of metastatic disease), the lesion was associated with xeroderma pigmentosum.

DISCUSSION

Malignant melanoma constitutes 1–2% of human malignancies in the United States and approximately 0.9% of deaths due to cancer in this country. However, in Everson and Cole's monograph (1) it accounted for 11% of spontaneous regressions reported at that time. Thus the incidence of spontaneous regression of malignant melanoma is fivefold to sixfold the expected incidence, which suggests that the phenomenon is more common in this disease and perhaps has a more significant role in its natural history than in other malignancies.

The data reported above, concerning the evidence for the phenomenon of spontaneous regression in its various phases in malignant melanoma, suggest the possibility that there may be a spectrum of such phenomena, which would include the following:

Halo Nevus

This could represent, as suggested by Lewis (12), a "forme fruste" of primary melanoma. That is a lesion undergoing the first changes of the "malignant intralesional transformation" process described by Clark et al. (78), in which the transformed cells are undergoing intense and successful attack by the host defense mechanisms.

Regression of Primary Melanoma

As a phenomenon in which a small area or cluster of cells of the primary undergoes regression, often with depigmentation, this may be a relatively frequent phenomenon (6). When the primary regresses completely, with or without concurrent development of metastatic disease, this might represent an intermediate degree of success of host protective mechanisms.

Regression of Established Metastatic Malignant Melanoma

This extremely rare phenomenon may represent the most striking clinical manifestation of host defense factors. A factor which is, for some yet unknown reason, delayed, so that rather than occurring early in the disease, as was the case with the "halo nevus" or with regression of primary tumor, it takes place only when metastatic disease has developed.

This sequence of the spontaneous regressive phenomenon in melanoma appears to be highly selective in character. That is to say, certain "clones" of cells in the primary lesion may undergo host attack, while other cells appear to grow unabated. Similarly, the entire primary may undergo host attack, while metastatic lesions continue to progress. This phenomenon of selectivity suggests that whatever the mechanism, whether immunologic or not, it is directed against specific features of the regressing tumor cells, which are not held in common with other cells in the tumor.

It is also of interest that there appear to be distinctly different types of regression. Those regressions of cutaneous melanoma, whether primary or metastatic, that are accompanied by a halo depigmentation may well represent a qualitatively different phenomenon from those that do not. In addition, the appearance of an infiltrate of chronic

inflammatory cells accompanying cutaneous regression is by no means universally noted and, in fact, has been thought by some to be an exception rather than a rule (57). On the other hand, McGovern (8), who emphasized the variability of the type of regression described above, thinks that the commonest type of pattern seen in regressing melanoma is that in which there is a dense infiltrate of lymphocytes associated with degenerating melanoma cells. Little (61) noted the inverse relationship between degree of infiltration of plasma cells and prognosis, but he also pointed out that such plasma cell infiltration may be a secondary result of ulceration of these lesions.

Attempts to relate the presence or absence of preexisting moles to the prognosis in malignant melanoma, or to the incidence of spontaneous regression, have been fruitless. Similarly, attempts to determine ultrastructural features of malignant melanoma have resulted in a fairly complex classification of four types of melanoma cells by Clark et al. (78). These fine structural features seem to be correlated with biologic behavior, but whether such fine structural changes will, in addition, characterize areas of spontaneous regression within a primary or metastatic melanoma is unclear. Similarly, light microscopic classification of melanoma cells by mitotic rate and by nuclear, nucleolar, or cytoplasmic features may in the future be helpful in understanding the mechanism of spontaneous regression.

Animal models of spontaneous regression such as that in Sinclair swine (122) may also contribute to our understanding of this process. Regression in this animal is accompanied by vitiliginous change, which is most reminiscent of the human counterpart.

APPENDIX: Case Report of Spontaneously Regressing Primary Melanoma

Mr. B. W. (New England Medical Center Hospital 12-7-554) was a 49-year-old, white, married male, who was first admitted to the hospital in June 1959 with a diagnosis of chronic alcoholism, Laennec's cirrhosis, acute gastritis, and a "blue nevus" on the mid-back. He stated at the time that this lesion had been present for several years. The lesion was noted to be 1–2 cm in diameter and was darkly pigmented; excision was recommended. However, this recommendation was not carried out, and the patient was not seen again until June 1963. At that time he reported that 2–3 years prior to his visit (?1960) his wife had expressed some dark material from the lesion, which "gave him no trouble thereafter." He further stated that approximately a month before being seen, he developed a lump in the skin of his right shoulder. However, no further medical procedure or diagnosis was made until December 1963, at which time he entered the hospital and was noted to have a blue-black telangiectatic lesion (about 2 cm) over the right shoulder and numerous subcutaneous nodules over the skin of the right forearm, posterior neck, and lower back. These lesions were movable and nontender. He was also noted to have an approximately 9-mm lesion in the mid-back, corresponding precisely to the location of the previously noted "blue nevus." This lesion was seen to be undergoing central necrosis and regression, accompanied by the presence of halo depigmentation measuring approximately 1.5 cm in diameter (fig. 1).

Chest X-ray demonstrated the presence of at least five round pulmonary densities compatible with metastatic tumor and fractures of the right fifth and left sixth ribs. An extrinsic mass in the anterior rectosigmoid was noted on barium enema examination. Biopsies were done on the

metastatic subcutaneous nodules, which showed amelanotic melanoma, and on the primary lesion, which showed histopathologic evidence of necrotic tumor cells together with an infiltrate of chronic mononuclear cells including lymphocytes and plasma cells. The patient was considered for laser-beam therapy but the consideration was rejected because of the presence of his visceral metastases. No further treatment was administered and in January 1964 he was admitted to another hospital and died from a subarachnoid hemorrhage, presumably secondary to hemorrhagic intracranial metastases of melanoma. A secondary cause of death was chronic alcoholism and Laennec's cirrhosis.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous regression of malignant melanoma. In *Spontaneous Regression of Cancer*. Philadelphia, Saunders, 1966, p 560
- (2) BIERMAN EO: Spontaneous regression of malignant disease. *JAMA* 170:1842-1850, 1959
- (3) CAPPELAERE P, DAMAILLE A, ADENIS L: L'exploration immunitaire des cancéreux. II. Immunité tumorale. *Lille Med* 18:179-201, 1973
- (4) FAUVET J, CAMPAGNE J, CHAVEY A, et al: Guérisons, régressions et remissions spontanées des cancers. *Rev Pratique* 10:2349-2384, 1960
- (5) FAUVET J, ROUJEAU J, PIET G: Les guérisons et régressions spontanées des cancers. *Rev Pratique* 14:2177-2180, 1964
- (6) MIHM MC, FITZPATRICK TB, LANE-BROWN MM, et al: Early detection of primary cutaneous malignant melanoma: A color atlas. *N Engl J Med* 289:989-995, 1973
- (7) LITTLE JH: Partial regression of primary cutaneous malignant melanoma. *Pathology* 3:62-70, 1971
- (8) MCGOVERN VJ: Melanoma: Growth patterns, multiplicity and regression. In *Melanoma and Skin Cancer* (McCarthy WH, ed.). Sydney, Blight, 1972, pp 95-106
- (9) MCGOVERN VJ, MIHM MC JR, BAILLY C, et al: The classification of malignant melanoma and its histologic reporting. *Cancer* 32:1446-1457, 1973
- (10) REESE AB, ARENAS AE, JONES IS, et al: Necrosis of malignant melanoma of the choroid. *Am J Ophthalmol* 69:91-104, 1970
- (11) EPSTEIN WL, FUKUYAMA K: In vitro culture of cloned hamster melanoma cells containing R-type virus. *Cancer Res* 33:825-831, 1973
- (12) LEWIS MG: Halo nevus: A frustrated malignant melanoma? *Lancet* 2:47-48, 1972
- (13) LANE N, LATTES R, MALM J: Clinicopathological correlations in a series of 117 malignant melanomas of the skin in adults. *Cancer* 11:1025, 1958
- (14) PACK GT, MILLER TR: Metastatic melanoma with indeterminate primary site (report of two instances, of long-term survival). *JAMA* 176:55-61, 1961
- (15) CHARALAMBIDIS PH, PATTERSON WB: A clinical study of 250 patients with malignant melanoma. *Surg Gynecol Obstet* 115:333-345, 1962
- (16) DAS GUPTA T, BOWDEN L, BERG J: Malignant melanoma of unknown primary origin. *Surg Gynecol Obstet* 117:341-345, 1963
- (17) SMITH JL, STEHLIN JS: Spontaneous regression of primary malignant melanoma with regional metastases. *Cancer* 18:1399-1415, 1965
- (18) MCGOVERN VJ: Melanoblastoma in Australia. Structure & Control of the Melanocyte. Berlin, Springer-Verlag, 1966, pp 312-316
- (19) NATHANSON L, HALL TC, VAWTER GF, et al: Melanoma as a medical problem. *Arch Intern Med* 119:479-492, 1967
- (20) NATHANSON L, NECHELES T, CLARK DA, et al: HL-A masking activity in malignant melanoma. *Behring Inst Mitt* 56:97-103, 1975
- (21) IJIMA S, WATANABE S, SHIMODA C: A case of melanoma associated with xeroderma pigmentosum. *Acta Dermatol (Japan)* 52:163-167, 1957
- (22) RONCHESI F: Spontaneous bleaching of melanotic freckles. *Arch Dermatol* 94:739-741, 1966
- (23) TODD DW, FARROW GM, WINKELMANN RK, et al: Spontaneous regression of primary malignant melanoma with regional metastases: Report of a case with photographic documentation. *Mayo Clin Proc* 41:672-676, 1966
- (24) MILTON GW, LANE-BROWN MM, GILDER M: Malignant melanoma with occult primary lesion. *Br J Surg* 54:651-658, 1967
- (25) PARACHE RM, EHARDOT C: Spontaneous local regression of a metastatic fatal melanoma. *Presse Med* 77:2059-2060, 1969
- (26) FARNSWORTH J: Regressing melanoma metastasizing to an oligodendroglioma. *Pathology (Australia)* 4:253-257, 1972
- (27) BRINCKER H, ANDERSON AP: Spontaneous regression of malignant tumors. *Ugeskr Laeger* 134:597-601, 1972
- (28) IKONOPISOV RK: Morphologic patterns of spontaneously regressing melanoma in relation to host immune reactions. In *Pigment Cell: Mechanisms in Pigmentation* (McGovern VJ, Russel P, eds.), vol 1. Basel, Karger, 1973, pp 402-409
- (29) SHIRAKAWA S, LUCE JK, TANNOCK I, et al: Cell proliferation in human melanoma. *J Clin Invest* 49:1188-1199, 1970
- (30) MOORE GE, GERNER RE: Malignant melanoma. *Surg Gynecol Obstet* 132:427-436, 1971
- (31) DALAND EM, HOLMES JA: Malignant melanomas. *N Engl J Med* 220:651, 1939
- (32) VOGLER WR, PERDUE GD, WILKINS SA JR: A clinical evaluation of malignant melanoma. *Surg Gynecol Obstet* 106:586-592, 1958
- (33) CADE S: Malignant melanoma. *Ann R Coll Surg Engl* 28:331-338, 1961
- (34) BLOCK GE, HARTWELL SW JR: Malignant melanoma: A study of 217 cases. *Ann Surg (suppl)* 154:88-98, 1961
- (35) PETERSON NC, BODENHAM DC, LLOYD OC: Malignant melanomas of the skin. *Br J Plast Surg* 15:49-97, 1962
- (36) MATHEWS FS: Melanosarcoma of shoulder and nodes about shoulder: Well after incomplete operation. *Ann Surg* 62:144, 1915
- (37) DALAND EM: Malignant melanoma (personal experience with 170 cases). *N Engl J Med* 260:453-459, 1959
- (38) PACK GT: Note on the experimental use of rabies vaccine for melanomatosis. *AMA Arch Derm Syph (Chicago)* 62:694, 1950
- (39) HIGGINS GK, PACK GT: Virus therapy in the treatment of tumors. *Bull Hosp Joint Dis* 12:379-386, 1951
- (40) LEVI JE, LEWISON EF: Malignant melanoma in a patient with ovarian agenesis. Case report of prolonged survival. *J Clin Endocrinol Metab* 12:901, 1952
- (41) SUMNER WC: Spontaneous regression of melanoma: A report of a case. *Cancer* 6:1040-1048, 1953
- (42) SUMNER WC, FORAKER AG: Spontaneous regression of human melanoma: Clinical and experimental studies. *Cancer* 13:79-81, 1960
- (43) ALLEN EP: Malignant melanoma, spontaneous regression after pregnancy. *Br Med J* 2:1067-1072, 1955
- (44) FOLEY W, COON WW: Unusual clinical course in patients with malignant melanoma. *Mich Med* 69:763-766, 1970
- (45) LEVISON VB: Spontaneous regression of a malignant melanoma. *Br Med J* 1:458-463, 1955
- (46) MALLESON N: Spontaneous regression of malignant melanoma. *Br Med J* 1:668-669, 1955
- (47) BOYD W: Spontaneous regression of cancer. *J Can Assoc Radiol* 8:45-54, 1957
- (48) STIDOLPH NE: Malignant melanoma regression of metastases after excision of primary. *Proc R Soc Med* 60:1, 1967
- (49) THE TH, EINBERGEN R, LAMBERTS HB, et al: Immune phagocytosis in vivo of human malignant melanoma cells. *Acta Med Scand* 192:141-144, 1972
- (50) DOYLE JC, BENNETT RC, NEWING RK: Spontaneous regression of malignant melanoma. *Med J Aust* 2:551-552, 1973
- (51) GOULD P: The pathology of melanotic growths in relation to their operative treatment (cited by Handley WS). *Lancet* 1:927, 1907
- (52) SWAN RH: The pathology of melanotic growths in relation to their operative treatment (cited by Handley WS). *Lancet* 1:927, 1907
- (53) BYRD BF JR, MCGANITY WJ: The effect of pregnancy on the clinical course of malignant melanoma. *South Med J* 47:196-203, 1954
- (54) TURNER FP, CRAWFORD AM: Malignant melanoma with brain metastasis: Case report with seven-year survival. *J Maine Med Assoc* 52:204, 1961
- (55) BESWICK IP, QVIST G: Spontaneous regression of cancer. *Br Med J* 2:930-931, 1963
- (56) EILBER FR, MORTON DL: Impaired immunologic reactivity and recurrence following cancer surgery. *Cancer* 25:362-367, 1970

- (57) BERG JW: Morphologic evidence for immune response to breast cancer: An historical review. *Cancer* 28:1453-1456, 1971
- (58) BOWDEN L, McNEER G: Personal communication cited by John W. Berg in a paper presented at the Second National Conference on Breast Cancer, Los Angeles, Calif, May 17-19, 1971
- (59) SCHEFFER CH, BINKHORST PG: Proceedings: Choroid melanoblastoma with signs of regression. *Ophthalmologica* 167: 433-434, 1973
- (60) FALK RE, MANN P, LANGER B: Cell-mediated immunity to human tumors. Abrogation by serum factors and non-specific effects of oral BCG therapy. *Arch Surg* 107:261-265, 1973
- (61) LITTLE JH: Histology and prognosis in cutaneous melanoma. In *Melanoma and Skin Cancer* (McCarthy WH, ed.). Sydney, Blight, 1972, pp 107-120
- (62) LLOYD OC: Regression of malignant melanoma as a manifestation of a cellular immune response. *Proc R Soc Med* 62: 543-547, 1969
- (63) EPSTEIN WL, SAGEBEIL R, SPITLER L, et al: Halo nevi and melanoma. *JAMA* 225:373, 1973
- (64) NICHOLLS EM: Development and elimination of pigmented moles and the anatomic distribution of primary malignant melanoma. *Cancer* 32:191-195, 1974
- (65) WAYTE DM, HELWIG EB: Halo nevi. *Cancer* 22:69-90, 1968
- (66) LERNER AB: On the etiology of vitiligo and gray hair. *Am J Med* 51:141-151, 1971
- (67) BOR S, FEIWEIL MB, CHANARIN I: Vitiligo and its aetiological relationship to organ-specific autoimmune disease. *Br J Dermatol* 81:83-88, 1969
- (68) KATZ A, Pigby JW: Malignant melanoma and dermatomyositis. *Can Med Assoc J* 93:1367-1369, 1965
- (69) OREN ME, HERBERMAN RB: Delayed cutaneous reactions to membrane extracts of human tumour cells. *Clin Exp Immunol* 9:45-51, 1971
- (70) BLUMING AZ, VOGEL GL, ZIEGLER JL: Delayed cutaneous sensitivity reactions to extract of autologous malignant melanoma: A second look. *J Natl Cancer Inst* 48:17-24, 1972
- (71) The TH: Some recent developments in human tumour immunology. *Folia Med Neerl* 15:279-290, 1972
- (72) HELLSTRÖM KE, HELLSTRÖM I: Immunity to neuroblastomas and melanomas. *Ann Rev Med* 23:19-38, 1972
- (73) HELLSTRÖM I, HELLSTRÖM KE: Some recent studies on cellular immunity to human melanomas. *Fed Proc* 32:156-159, 1973
- (74) MORTON DL, EILBER FR, MALMGREN RA: Immune factors in human cancer: Malignant melanomas, skeletal and soft tissue sarcomas. *Prog Exp Tumor Res* 14:25-42, 1971
- (75) CLARK DA, NATHANSON L: Cellular immunity in malignant melanoma. In *Pigment Cell: Mechanisms in Pigmentation* (McGovern VJ, Russel P, eds.), vol 1. Basel, Karger, 1973, pp 350-359
- (76) CLARK DA, NECHELES T, NATHANSON L, et al: Apparent HL-A5 deficiency in malignant melanoma. *Transplantation* 15:326-328, 1973
- (77) CLARK DA, NECHELES TF, SILVERMAN E, et al: Apparent HL-A deficiency in human malignant melanoma. II. HL-A masking activity of patients with progressing disease. *Isr J Med Sci* 10:836-846, 1974
- (78) CLARK WH, HEGGELER B, BRETTON R: Electron microscope observations of human cutaneous melanoma correlated with their biologic behavior. In *Melanoma and Skin Cancer* (McCarthy WH, ed.). Sydney, Blight, 1972, pp 121-141
- (79) MUKHERJI B, FLOWERS A, NATHANSON L, et al: A hetero-transplantation model for human malignant melanoma. *Cancer Res* 34:43-46, 1974
- (80) MUKHERJI B, NATHANSON L, CLARK DA: Studies of humoral and cell-mediated immunity in human melanoma. *Yale J Biol Med* 46:681-692, 1973
- (81) NATHANSON L: Regression of intradermal malignant melanoma after intraslesional injection of *Mycobacterium bovis* strain BCG. *Cancer Chemother Rep* 56:659-665, 1972
- (82) NATHANSON L, CLARK DA: In vitro assay of cell-mediated immunity in BCG therapy of malignant melanoma: A preliminary report. *Natl Cancer Inst Monogr* 39:221-224, 1973
- (83) WYBRAN J, SPITLER LE, FUNDENBERG HH: Rosette-forming cells and melanoma. *Clin Res* 21:665, 1973
- (84) BURDICK KH: Malignant melanoma treated with vaccinia injections. *Arch Dermatol* 82:188-438, 1960
- (85) MILTON GW, LANE-BROWN MM: The limited role of attenuate smallpox virus in the management of advanced malignant melanoma. *Aust NZ J Surg* 35:286-293, 1966
- (86) HUNTER-CRAIG I, NEWTON KA, WESTBURG G, et al: Uses of vaccinia vaccine in the treatment of metastatic malignant melanoma. *Br Med J* 2:512-516, 1970
- (87) EASOM HA: Sympathetic ophthalmia associated with malignant melanoma. *Arch Ophthalmol* 70:786-790, 1963
- (88) MACKIE RM, CARFRAE DC, COCHRAN AJ: Assessment of prognosis in patients with malignant melanoma. *Lancet* 2:455-456, 1972
- (89) WHITE LP: The role of natural resistance in the prognosis of human melanoma. *Ann NY Acad Sci* 100:115-126, 1963
- (90) STEPHENSON HE JR, DELMEZ JA, RENDEN DI, et al: Host immunity and spontaneous regression of cancer evaluated by computerized data reduction study. *Surg Gynecol Obstet* 133:649-655, 1971
- (91) SADOFF L, WINKLEY J, TYSON S: Is malignant melanoma an endocrine-dependent tumor? *Oncology* 27:244-257, 1973
- (92) WHITE LP, LINDEN G, BRESLOW L, et al: Studies on melanoma: The effect of pregnancy on survival in human melanoma. *JAMA* 177:235-241, 1961
- (93) KLAUS SN, SNELL RS: The response of mammalian epidermal melanocytes in culture hormones. *J Invest Dermatol* 48:352-360, 1967
- (94) ORTH DN: Establishment of human malignant melanoma clonal cell lines that secrete ectopic adrenocorticotropin. *Nature* 242:26-28, 1973
- (95) BRAUNSTEIN GD, VAITUKAITIS JL, CARBONE PP, et al: Ectopic production of human chorionic gonadotrophin by human neoplasia. *Ann Intern Med* 78:39-45, 1973
- (96) FLAX H, SALIH H, WESTBURY G: Hormone therapy in malignant melanoma. *Lancet* (letter to the editor), July 21, 1973
- (97) JOHNSON RD, BISEL H, ANDREW N, et al: Phase I clinical study of 6 α -methyl-pregn-4-ene-3,11,20-trione. *Cancer Chemother Rep* 50:571-573, 1966
- (98) WIGBY PE, METZ MH: Striking regression of generalized subcutaneous and visceral metastases of malignant melanoma (melanoblastoma) following intensive high voltage roentgen irradiation of the pituitary gland. *Am J Roentgenol* 41:415-424, 1939
- (99) HOWES WE: Removal of testes in treatment of melanoma. *JAMA* 123:304-309, 1943
- (100) PACK GT, SCHARNAGEL IM: The prognosis for malignant melanoma in the pregnant woman. *Cancer* 4:324-330, 1951
- (101) SHIMKIN MB, BOLDREY EB, KELLEY KH, et al: Effects of surgical hypophysectomy in a man with malignant melanoma. *J Clin Endocrinol Metab* 12:439-444, 1952
- (102) CHIAN LT, WILGRAM GF: Tyrosinase inhibition: Its role in suntanning and in albinism. *Science* 155:198-200, 1967
- (103) FRENK E, OTT F: Evaluation of the toxicity of the monethyl ether of hydroquinone for mammalian melanocytes and melanoma cells. *J Invest Dermatol* 56:108-113, 1971
- (104) HOCHSTEIN P, COHEN G: The effects of various depigmentation agents on malignant melanoma in cell culture and in vivo. *Ann NY Acad Sci* 100:876-886, 1963
- (105) MCGREGOR BC, KATZ HI, DOE RP: Vitiligo and multiple glandular insufficiencies. *JAMA* 129:724-725, 1972
- (106) BURDICK KH, HAWK WA: Vitiligo in a case of vaccinia virus treated melanoma. *Cancer* 17:708-713, 1964
- (107) DEMOPOULOS HB, GERVEN MA, GAGDOYAN H: Selective inhibition of growth and respiration of melanomas by tyrosinase inhibitors. *J Natl Cancer Inst* 35:823-827, 1965
- (108) DEMOPOULOS HB: Effects of reducing the phenylalanine-tyrosine intake of patients with advanced malignant melanoma. *Cancer* 19:657-664, 1966
- (109) VOORHEES ML: Effect of alpha-methyl-p-tyrosine on 3,4-dihydroxyphenylalanine (DOPA) excretion of hamsters with melanotic melanoma. *Cancer Res* 28:452-454, 1968
- (110) MISHIMA Y: Lysosomes in melanin phagocytosis and synthesis. *Nature* 216:67, 1967
- (111) BULLOUGH WS, LAURENCE EB: Melanocyte chalone and mitotic control in melanomata. *Nature* 220:137-138, 1968
- (112) SEIJI M, NAKANO H, AKIBA H, et al: Inhibition of DNA and protein synthesis in melanocytes by a melanoma extract. *J Invest Dermatol* 62:11-19, 1974
- (113) FOLKMAN J, MERLER E, ABERNATHY C, et al: Isolation of a tumor factor responsible for angiogenesis. *J Exp Med* 133:275-288, 1971
- (114) BREM S, COTRAN R, FOLKMAN J: Tumor angiogenesis: Quantitative method for histologic grading. *J Natl Cancer Inst* 48:347-356, 1972
- (115) EPSTEIN JH, EPSTEIN WL, NAKAI T: Production of melanomas from DMB-A-induced "blue nevi" in hairless mice with ultraviolet light. *J Natl Cancer Inst* 38:19-30, 1967

- (116) LEE JA, MERRIL JM: Sunlight and the etiology of malignant melanoma: A synthesis. *Med J Aust* 2:846-851, 1970
- (117) GHADIALLY FN: Trauma and melanoma production. *Nature* 211:1199, 1966
- (118) ANDERSON D: Clinical characteristics of the genetic variety of cutaneous melanoma in man. *Cancer* 28:124-125, 1971
- (119) WALLACE DC, BEARDMORE GL, EXTON LA: Familial malignant melanoma. *Ann Surg* 177:15-20, 1973
- (120) KIRSCH N: Malignant melanoma developing in a tattoo. *Int J Dermatol* 11:16-20, 1972
- (121) STJERNSWÄRD J, LEVIN A: Delayed hypersensitivity-induced regression of human neoplasms. *Cancer* 28:628-640, 1971
- (122) MILLIKAN LE, BOYLON JL, HOOK RR, et al: Melanoma in Sinclair swine: A new animal model. *J Invest Dermatol* 62:20-30, 1974

ADDENDUM

Since this manuscript was prepared the author, together with Dr. Leo Stolbach of the Pondville Hospital, Walpole, Massachusetts, has seen another patient who exhibited spontaneous regression of metastatic melanoma.

Mrs. S. is a 58-year-old, white housewife and mother, who first noted a mole growing on her right foot sometime in late 1973 or early 1974. In March 1974, the lesion was excised and found to be a primary nodular melanoma (Clark level II-III); the lesion was widely excised with a right radical groin dissection because of palpable right inguinal adenopathy. The nodes were negative for tumor. Liver scan at that time showed a slight hepatomegaly without evidence of

discrete lesion. Regan isoenzyme and HCG were negative. Serum proteins (mg/ml) were total protein, 8.2; albumin, 4.8; alpha I, 4.8; alpha II, 0.7; beta 1.3; gamma, 1.3; IgG, 13.8; IgA, 4.6; and IgM, 2.7. Past medical history is of some interest in that the patient's sister and aunt died of cancer of unknown primary origin. Detailed studies of humoral and cellular immunity to melanoma antigens are currently in progress.

In October 1974, she developed a recurrent pigmented spot on her right groin, near the node dissection line, and another pigmented lesion over the anterior lower thigh. Both lesions were excised in November 1974 and demonstrated recurrent melanoma. A "foreign body giant cell reaction" was present in one of these lesions.

In January 1975, two more lesions were removed, one near the excision and one from the anterior thigh area; both demonstrated melanoma. All of these recurrences were intradermal.

She was referred to Dr. Leo Stolbach at the Pondville Hospital on January 13, 1975. On January 20, 1975, two new satellite lesions appeared. Both of these measured up to 0.3 cm in diameter and gradually over the next 4-5 weeks became flatter and smaller, without demonstrating any clear-cut, deeply pigmented halo. Work-up at this time showed normal brain scan, hemogram, normal intravenous pyelogram, and a positive intradermal skin test to purified protein derivative (negative skin tests to *Candida* extract, mumps, and streptokinase/streptodornase). Biopsy of one of the regressing lesions on May 30, 1975, showed "pigment-laden macrophages present in a circumscribed area of the upper dermis. There is no evidence of malignancy."

At present, the patient has one intradermal lesion that has failed to regress completely but that has not increased in size (6/3/75).



FIGURE 1.—Primary lesion showing halo depigmentation and central area of necrosis and spontaneous regression.

DISCUSSION

L. Nathanson: It has been suggested by Lewis that patients with halo nevi have circulating antibodies to melanoma cells, which can be demonstrated by immunofluorescence, as is the case for patients with melanoma. Halo nevi may represent a form, if you will, of a primary malignant melanoma. A halo nevus may be a primary lesion that never undergoes the "malignant intralesional transformation," which Wallace Clark has emphasized; and if one thinks in those terms, which admittedly are somewhat speculative at the moment, one may think of a sequence of possible events. The halo melanoma may be the one that is attacked early by immunologic host defense mechanisms. The primary melanoma may be attacked partially and therefore undergo some areas of spontaneous regression and depigmentation; or it may be attacked successfully, in which case the primary is totally eradicated, even though the metastases continue to develop; or third in the spectrum of events, a large mass of metastatic lesions may be successfully attacked and successfully defended, if you will, by these host mechanisms. So there may well be a spectrum of events in terms of the ability with which the host defense mechanisms cope with the disease, and we may be seeing a whole variety of such phenomena.

H. C. Nauts: I was wondering whether Dr. Nathanson or any of those present are aware that immunotherapy has been used in malignant melanoma rather successfully. Bacterial toxin therapy has been used in quite a large number of well-documented cases, and the results are quite impressive. We published a monograph on this subject years ago. Since then we have seen an additional patient worthy of comment here. He was treated with surgery and immunotherapy for lung metastasis from melanoma. During the early phase of his immunotherapy, he developed three more rather large lesions, which regressed over a period of months with intralesional injections of bacterial toxins, and the patient is now apparently clinically well, 5½ years after he had lung metastasis.

Nathanson: Yes. In fact, I think, Miss Nauts, you sent me some of your literature and I appreciate it very much. I agree with the interesting nature of these early observations. In fact, I wanted to emphasize that one of the most interesting things about melanoma is that it provides an unusual tumor in which there appears to be a manipulatable method of, if not entirely regularly at least occasionally, inducing a type of "spontaneous regression" by a variety of different immunoadjuvant techniques, so that this perhaps makes it unusually susceptible to further study in terms of the mechanisms involved in such a process.

II.

CLINICAL AND EXPERIMENTAL MECHANISMS OF ACTION

Regression of Cancer Following Surgery

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SUMMARY—Postsurgical tumor regressions are rare but well-recognized entities. The causes of such phenomena are unknown but probably are multiple. Regression of any tumor must ultimately come about by changes in the host-tumor interaction which suddenly becomes unfavorable for tumor growth. This is likely a manifestation of changes in the immunocompetence of the host. There are many variables influencing the host-tumor interaction, and the study of tumor immunology is still in its infancy. It is through the exploration and investigation of basic mechanisms of the immunology of cancer that the most potentially fruitful associations between tumor growth or regression and surgical manipulations may be made and applied to the treatment of cancer patients. *Natl Cancer Inst Monogr* 44: 81-84, 1976.

Spontaneous regression of cancer is a rare phenomenon. Any association between tumor regression and surgery can be made only by indirect inferences based on reviews of patients' histories. A total of 107 cases of well-documented tumor regression following noncurative surgical therapy was collected from the world's literature through 1973. The compilation of cases, edited by Everson and Cole (1), was relied upon heavily. However, associating the effects of surgery with the phenomenon of regression was difficult in many cases because of the concomitant use of adjunctive treatment modalities, such as radiation or chemotherapy. Consequently, a set of criteria was established for classifying a case as a documented regression: 1) major surgical procedure preceding the regression, with initial operative intent of exploration for possible cure; 2) inadequate surgical treatment for cure, with local tumor or distant metastases left unresected; 3) histologic confirmation of malignancy and of the tumor type; 4) complete tumor regression within 12 months of surgery to a clinically disease-free state; 5) maintenance of a disease-free state for at least 6 months; 6) clinical and laboratory confirmation of regression of the primary tumor or of metastases; and 7) no adjunctive therapy in any form.

A CASE REPORT

A 38-year-old Caucasian housewife first sought medical attention in 1953, when a 1×1-cm black mole was noted on the lateral aspect of the right calf, appearing just after her second pregnancy. The lesion remained unchanged until 1958, when the mole enlarged and started bleeding

during the 5th month of her third pregnancy. Biopsy showed malignant melanoma. Wide local excision and skin graft were performed, and pathologic evaluation showed no residual tumor in the site of biopsy.

The patient did well until late 1960, when an enlarging mass developed in the lateral mid-thigh region of the right leg. Biopsy showed metastatic melanoma, and a right radical groin dissection was performed. Three inguinal lymph nodes were positive for metastatic melanoma. The patient developed a postoperative wound infection, which required drainage and several weeks of granulation to achieve healing.

The patient remained free of disease until early 1964, when several cutaneous nodules developed in the medial right thigh and progressed in size over a 3-month period. Biopsy of the subcutaneous nodules showed metastatic melanoma. The patient underwent an exploratory staging laparotomy with the finding of pea-sized pigmented liver nodules showing melanoma on biopsy. Multiple intra-abdominal and retroperitoneal lymph node biopsies were negative for tumor.

Because of the visceral metastatic disease, no further surgical therapy was offered. The patient was recommended for treatment with phenylalanine mustard and triethylene-thiophosphoramide (thio-TEPA). While chemotherapy was being arranged, the patient noted regression of the tumor nodules in the right thigh. Within 3 weeks, all signs of cutaneous melanoma had completely disappeared. Consequently, no treatment was given, and the patient was followed closely, with no evidence of disease for the next 4 years.

The patient underwent cervical conization in 1968 for carcinoma in situ. An abdominal hysterectomy was performed, and a thorough abdominal exploration failed to show any evidence of melanoma. A liver biopsy was normal.

The patient has since been followed at yearly intervals and has remained free of evidence of recurrent melanoma 16 years after excision of the primary lesion and 12 years after spontaneous tumor regression.

SUMMARY OF POSTSURGICAL CANCER REGRESSION

With the above case report, 108 instances of postsurgical cancer regression have been documented (table 1). Tumor regression appears to take place most often in unusual types of cancers. Although they form less than 8% of all cancers in the general population, four tumor types (renal carcinoma, melanoma, neuroblastoma, and choriocarcinoma) constitute almost two-thirds of all cases of postsurgical tumor regression (table 2). Only 13 patients represented instances of spontaneous regressions of the common tumor types: gastrointestinal, genital, breast, and lung. Among the patients with cases of regression following surgery, 56 were males and 52 were females (table 3). The ages at regression ranged from 2 months to 81 years, with the age distribution of regressed tumors corresponding closely with the expected peak age of incidence for the

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TABLE 1.—*Documented cases of tumor regression after surgery*

Tumor type	Number of cases
Renal	28
Melanoma	13
Neuroblastoma	13
Choriocarcinoma	11
Sarcoma, bone and soft tissue	11
Bladder	10
Colon	4
Gastric	4
Ovarian	3
Pancreatic	2
Uterine	2
Breast	1
Testicular	1
Thyroid	1
Metastatic, unknown primary	4
Total	108

TABLE 2.—*Frequency of various tumor types regressing after surgery*

Tumor type	Percentage of regressions	Percentage of all cancers
Urinary tract	35	5.1
Melanoma	12	1.4
Neuroblastoma	12	0.2
Choriocarcinoma	10	0.8
Sarcoma	10	1.0
Gastrointestinal	9.3	21
Endocrine	4.9	6.1
Genital	2.0	14
Breast	0.9	12
Unknown primary	3.9	4.1

TABLE 3.—*Sex distribution of tumor regression after surgery*

Tumor type	Number of cases	Males	Females
Urinary tract	38	30	8
Melanoma	13	4	9
Neuroblastoma	13	8	5
Choriocarcinoma	11	1	10
Sarcoma	11	4	7
Gastrointestinal	10	5	5
Endocrine	5	2	3
Genital	2	0	2
Breast	1	0	1
Unknown primary	4	2	2
Total	108	56	52

various tumor types (table 4). Most regressions of neuroblastoma occurred in patients under 1 year of age, the peak of neuroblastoma incidence, and most regressions of carcinoma of the urinary tract occurred during the 50- to 59-year age range. Insufficient data was available on race, geographic distribution, or other epidemiologic factors.

POSSIBLE CAUSATIVE FACTORS

It is not possible to draw significant conclusions or meaningful correlations from a small series of case histories. However, certain recurrent patterns deserve consideration of possible roles in influencing tumor regression. Those patterns include: 1) endocrine factors, 2) psychologic factors, 3) trauma and stress, 4) diversion of urinary and fecal streams, and 5) fever and infection.

Endocrine Factors

The phenomenon of acceleration of melanoma with pregnancy was recognized early (2) and was suggested to be a result of hormone stimulation. Spencer (3) suggested that the growth rate of melanoma is related to hormone influences, since periods of elevated hormone activity, such as puberty, are frequently accompanied by excitation of tumor growth. In an analysis of 224 cases of tumor regression, Stephenson and his co-workers (4) found pregnancy or other significant hormone relationships associated with 45 instances. Other researchers (5, 6) noted increased survival rates in premenopausal women with melanoma and an increased frequency of activation of disease after the cessation of menses. Several other types of hormonally responsive tumors are known, most notably mammary, ovary, and prostate.

In regard to tumor regression after surgery, endocrine influences must be evaluated as possible contributing factors, since operative stress and anesthesia have profound effects on hormone balance. Perioperative medications also might alter hormone relationships and thereby influence tumor growth and regression.

Psychologic Factors

The psychologic effects of hospitalization and surgery may be important in tumor regression. When the trauma of hospitalization is compounded by a diagnosis of cancer and by surgery, which might or might not be curative, the emotional effects on some patients may be overwhelming. Psychologic factors can considerably alter an individual's hormone and metabolic balance by a variety of mechanisms: by corticoid or catecholamine release; by direct neuroendocrine influences; by alterations in behavior patterns with secondary metabolic influences, such as anorexia with depression and subsequent cachexia; or by mechanisms not yet elucidated. These influences may alter host responsiveness such that certain tumors might find a metabolic environment that was no longer suitable for growth. Hence through indirect metabolic pathways, emotional aspects of certain cancer patients may influence growth or regression of their tumors. Certainly, "faith healers" and "miracle cancer cures" might derive benefit from these psychologic effects.

Trauma and Stress

The endocrine and metabolic responses to trauma and stress have been well described (7, 8). Surgical procedures result in increases in circulating steroids as well as catecholamines and various metabolites. Steroids have been shown

TABLE 4.—Age distribution of tumor regression after surgery

Tumor type	Number of cases	Age of patients (yr)									
		<1	1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	≥80
Urinary tract	38	—	—	—	—	2	4	20	10	2	—
Melanoma	13	—	—	—	2	5	5	1	—	—	—
Neuroblastoma	13	11	2	—	—	—	—	—	—	—	—
Choriocarcinoma	11	—	—	—	7	2	1	1	—	—	—
Sarcoma	11	3	2	2	2	2	—	—	—	—	—
Gastrointestinal	10	—	—	—	—	—	3	5	2	—	—
Endocrine	5	1	—	—	1	1	1	—	—	—	1
Genital	2	—	—	—	—	—	—	—	1	1	—
Breast	1	—	—	—	—	—	—	—	1	—	—
Unknown primary	4	—	—	—	—	1	3	—	—	—	—
Total	108	15	4	2	12	13	17	27	14	3	1

to cause tumor regressions in a variety of animal systems (9), and certain human neoplasms are known to exhibit significant hormone responsiveness in terms of their growth patterns.

Because of the complexities of hormone and metabolic interrelationships, it is difficult to assess critically the actual degree of influence of stress-induced physiologic changes on neoplasm growth. However, it would be naive to ignore the potential influences of surgery-induced hormone changes on the development, maintenance, or regression of certain tumors.

Diversion of Urinary and Fecal Streams

Of 10 cases of bladder carcinoma that spontaneously regressed after surgery, 9 did so after urinary diversion. Regression of bladder carcinoma after diversion has led to speculation that it might be due to removal of some carcinogenic agent in the urine (1, 10). Though tenable, such a hypothesis has little experimental verification, since no carcinogenic agent has been isolated from the urine of patients with bladder tumors. However, the lack of continuing tumor irritation by a constant urine flow after diversion might be a contributing factor to regression.

A similar incidence of regression of tumors of the colon and rectum has been noted after surgical diversion of the fecal stream. Of the 4 collected cases of postsurgical colon cancer remissions, 2 regressed after the creation of diverting colostomies. Again, a possible etiologic factor in remission is the removal of chronic irritation and trauma to the tumor by constant exposure to the fecal stream. Surgical correction of a source of constant irritation to an already existing tumor might be considered an adjunct to achieving local tumor control.

Infections and Fever

Of 108 patients with spontaneous tumor regressions following surgery, 14 had significant infectious surgical complications, including frank sepsis with abscess formation (8), wound drainage and fistula formation (3), and persistent fever (3). The series is too limited and the cases are too unrelated to warrant drawing any significant conclusions. However, an association between surgical infections and improved prognosis of tumors has been re-

peatedly suggested. In a computerized compilation of data on case reports of spontaneous tumor regression, Stephenson and associates (4) found that of 224 cases, 62 patients (28%) were reported to have had an infection or a persistent temperature elevation prior to regression. In an early study, Rohdenberg (11) reviewed 302 reports of probable spontaneous cancer regression, noting a particularly frequent association with infections such as erysipelas, pneumonia, and tuberculosis. Takita (12) reported an increased survival of patients who developed empyema after pulmonary resection for bronchogenic carcinoma, finding a 54% 5-year survival for the patient group that experienced postoperative infection. Sensenig and associates (13) reported a 57% 5-year survival in patients with empyema following surgery for lung cancer.

The apparent association between an infectious or inflammatory response and impaired survival of patients with a variety of tumors suggests that the inflammatory response by some mechanism enhances host resistance to the tumor. This association suggests immunologic stimulation. Any infection stimulates the lymphoreticular system and causes an overall enhanced immunologic responsiveness. Such a generalized immune stimulation might result in mobilization of host defense mechanisms, thus contributing to tumor regression.

Other Possible Factors

Exposure of the patient to various agents during diagnostic evaluation in the hospital can also alter host-tumor relationships. During a typical diagnostic evaluation at a cancer center or a university hospital, a tumor patient undergoes a wide variety of radiologic, radioisotopic, and pharmacologic tests, all of which expose the patient to potential agents that might alter tumor growth in certain sensitive individuals. For example, a patient being staged at the National Institutes of Health for malignant melanoma undergoes a comprehensive series of X-rays: metastatic bone survey, chest X-ray with lung tomograms, intravenous pyelogram, upper gastrointestinal series, and barium enema. The patient also undergoes a series of radio-nuclide scans: brain, bone, liver-spleen, lung, total body gallium, and total body bleomycin. Such basic diagnostic tests effect a radiologic exposure averaging approximately 3-5 rads and a radioisotope load of approximately 10 mCi

of gamma emitters. Patients with other tumors may receive additional diagnostic X-rays or scans, such as sinus tomograms for facial tumors, arteriography for intra-abdominal sarcomas, fluoroscopy for esophageal or gastric cancers, and breast and bone marrow scans for breast cancers, all adding to the total radiation exposure.

In addition, nutritional factors may be altered. Patients may receive improved preoperative nutrition by a hospital diet or by supplementary intravenous hyperalimentation. On the other hand, they may have a temporary negative nutritional balance because of repeated fastings and X-ray bowel preparations. Preoperative antibiotics and other pharmacologic agents must be considered as possible sources of affecting host-tumor interaction. Obviously, all examples considered individually cannot be demonstrated to cause significant alterations in the growth of a patient's tumor; however, they serve to point out the numerous possibilities of varying agents to which a patient might be exposed, which might alter the host-tumor relationship. The true significance of exposure to agents during a diagnostic evaluation and the possibility of association of the agents with tumor regression can at present be only a subject of speculation.

Operative manipulations, perioperative support, and postoperative complications are potential sources of influence on tumor growth and development. At surgery, attempts at resection of inoperable cancers can disseminate tumor cells into blood vessels, into lymphatics, or transcoelomically. Such cell dissemination might produce a sudden tumor-antigen load on a patient of sufficient magnitude to stimulate a host antitumor immune response. Surgical debulking has long been considered beneficial in certain patients with unresectable tumors. Debulking presumptively acts by reducing overall tumor load to allow host defenses to act more efficiently against the remaining cancer cells. Indeed, debulking is used frequently as an adjunctive measure to chemotherapy, to allow antineoplastic agents to act more efficiently against fewer total tumor cells. Devascularization of tumor mass must also be considered a possibility in postsurgical tumor regression with alterations in tumor blood supply from operative manipulations possibly causing slow ischemic regression. Removal of all or part of certain primary tumors may cause regression of distant metastases. This phenomenon is particularly prevalent among renal carcinomas and is likely mediated by trophic factors produced by the primary, which maintain the growth of the metastases.

Adjunctive factors to surgery might influence host-tumor responses. The most obvious of such factors include the anesthetic agents, with their known capacities to alter

immunologic responsiveness (14). Transfused blood and blood products might cause certain changes in host-tumor balance, perhaps by passive transfer mechanisms. Likewise, operative antibiotics, irrigating solutions, and exposure of the operative field to possible contaminants must be considered as potential sources of influence on subsequent tumor growth.

Postoperative complications open an entire class of potential agents altering tumor growth and survival. Sepsis, wound abscesses, wound separations, and fistulas have been associated with some documented reports of tumor regression. The mechanisms of action are unknown but probably represent enhancement of host response against the tumor, possibly through a generalized inflammatory process promoting immune stimulation. The possibility of tumorcidal bacterial toxins resulting from infections must also be considered. Pharmacologic agents used during the postoperative period, principally antibiotics, also might have some influence in tumor growth.

REFERENCES

- (1) EVERSON TC, COLE WH: Spontaneous Regression of Cancer. Philadelphia, Saunders, 1966, pp 6-89
- (2) PACK GT, SCHARNAGEL IM: The prognosis for malignant melanoma in the pregnant woman. *Cancer* 4:324-336, 1951
- (3) SPENCER RP: Malignant melanoma. *N Engl J Med* 253:18-29, 1955
- (4) STEPHENSON HE, DELMEZ JA, RENDEN DI, et al: Host immunity and spontaneous regression of cancer evaluated by a computerized data reduction study. *Surg Gynecol Obstet* 133:649-671, 1971
- (5) WRIGHT RB, CLARK DH, MILNE JA: Malignant cutaneous melanoma: A review. *Br J Surg* 40:360-374, 1953
- (6) WHITE LP: The role of natural resistance in the prognosis of human melanoma. *Ann NY Acad Sci* 100:115-129, 1963
- (7) STONER HB, THRELFALL CJ (eds.): *The Biochemical Response to Injury*. Springfield, Ill., Charles C Thomas, 1960, pp 19-38
- (8) LEVENSON SM, PULASKI EJ, DELGUERCIO LR: Metabolic changes associated with injury. In *Physiologic Principles of Surgery* (Zimmerman LM, Levine R, eds.). Philadelphia, Saunders, 1964, pp 122-184
- (9) VOLLMER EP: A viewpoint on animal tumors as test system for steroids. *Proc First Internat Cong Horm Ster* 2:351-366, 1965
- (10) EVERSON TC: Spontaneous regression of cancer. *Ann NY Acad Sci* 114:721-740, 1964
- (11) ROHDENBERG GL: Fluctuations in the growth rate of malignant tumors in man, with especial reference to spontaneous regression. *J Cancer Res* 3:193-221, 1918
- (12) TAKITA H: Effect of postoperative empyema on survival of patients with bronchogenic carcinoma. *J Thorac Cardiovasc Surg* 59:642-668, 1970
- (13) SENSENIG DM, ROSSI NP, EHRENSHAFT JL: Results of the surgical treatment of bronchogenic carcinoma. *Surg Gynecol Obstet* 116:279-296, 1963
- (14) JUBERT AV, HERSH EM, MCBRIDE CM: Effects of surgery, anesthesia, and intraoperative blood loss on immunocompetence. *J Surg Res* 15:399-414, 1973

Immunotherapy in Malignant Melanoma^{1,2}

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SUMMARY—Accumulated evidence suggests that human neoplasms contain antigens that elicit humoral and cellular immunity in the immunocompetent host. A recent summary report showed that Stage II melanoma patients with metastases to regional lymph nodes had a lowered incidence of recurrence and a higher incidence of survival following surgery and postoperative BCG immunotherapy. To verify these findings, clinical trials are now under way in which we randomized melanoma patients into groups to compare treatment by surgery alone with surgery and BCG only, or surgery and BCG in combination with allogeneic melanoma cell vaccine. Serum samples from each patient are monitored by *in vitro* techniques to define those methods which best correlate to clinical course. Hopefully, such correlations can be used to monitor response to immunotherapy before disease is clinically apparent. Although immunotherapy does not cause regression of far advanced disease, it undoubtedly will be beneficial against subclinical, microscopic tumor.—*Natl Cancer Inst Monogr* 44: 85–86, 1976.

During the past several years it has become increasingly apparent that immunologic factors are important in host-tumor defense mechanisms. The evidence accumulated to date suggests that human neoplasms contain antigens that elicit humoral and cellular immunity in the host. Furthermore, there is good evidence that the general immunologic competence of the host is important in the progression of human cancer. It seems reasonable, therefore, that the principles of tumor immunology should be applied to studies in the areas of etiology, diagnosis, and treatment of human cancer.

It is clear that surgical extirpation and occasionally radiation therapy can control the primary tumor. However, many tumors disseminate early and therefore some form of systemic surgical adjuvant therapy is indicated. We believe that the ideal time to initiate such a systemic antitumor regimen is in the early postoperative period when the tumor volume is small and the disease is subclinical. The requirements for such a systemic antitumor adjunct to surgery, as we see them, are as follows: 1) The adjuvant must be relatively nontoxic and free of serious side effects; 2) it must be highly specific for the tumor cells; 3) there must be some way to monitor therapy when the disease is subclinical; and 4) the treatment must have sound theoretical and experimental support.

There is substantial evidence to suggest that immunotherapy may fulfill these requirements. Pilot studies have shown that immunotherapy is relatively nontoxic and well tolerated (1). In addition, immune mechanisms are quite specific, and there are several promising *in vitro* tests that may be capable of monitoring host response to therapy when the disease is subclinical and cannot be monitored by standard clinical techniques. There is, of course, a large body of experimental data that suggests that immune mechanisms are important in the tumor-host relationship.

For several years we have been evaluating immunotherapy as a treatment modality in patients with malignant melanoma. Our initial efforts were directed toward the intralesional injection of intradermal metastases with BCG, and subsequently we began to use immunotherapy as an adjunct to surgery in patients with a high risk of recurrence. Forty-five patients have been treated with intratumor injections of BCG for metastatic disease; 36 had intransient intradermal metastases and 9 had subcutaneous or visceral metastases. Thirty-six patients with intradermal metastases have been treated over the last 7 years. A total of 754 melanoma nodules were injected in these 36 patients, and 91% of these underwent complete rejection. In addition, 17% had regression of the uninjected nodules (1). However, only those patients who were immunocompetent as judged by delayed cutaneous hypersensitivity to dinitrochlorobenzene had regression of the uninjected lesions (2). Patients with subcutaneous or visceral metastatic disease had a poor response following direct injection of BCG. Response to intralesional BCG injection appeared to correlate with 1) the location of the tumor (intradermal metastases responding best), 2) the immunocompetence of the patient, and 3) the amount of tumor present. Patients with large distant metastases did not respond well. It is our present opinion that intralesional BCG immunotherapy is the treatment of choice for immunocompetent patients with melanoma limited to the skin.

Having demonstrated the effectiveness of BCG immunotherapy in these patients, we began to investigate its usefulness as an adjunct to primary surgery for regional melanoma. This study began as a nonrandomized, phase I trial to determine whether BCG could be administered with acceptable toxicity and to look for evidence of therapeutic effect. For our purposes we consider Stage I melanoma to be disease confined to the primary site. In Stage II, the disease involves the regional lymph nodes, and Stage III is disease dissemination beyond the regional lymph nodes. In this study, patients with Stage II disease were started on BCG immunotherapy given intradermally by the time technique, beginning 3–4 weeks after regional lymphadenectomy (3). They received weekly BCG injections for 3 months and every 2 weeks thereafter.

Recurrence and survival rates were analyzed by the actuarial life-table method in order to include all patients treated to date (text-figs. 1, 2). The overall recurrence rate for the entire group of 67 patients is lower at all points in time in comparison with 34 Stage II patients, who were

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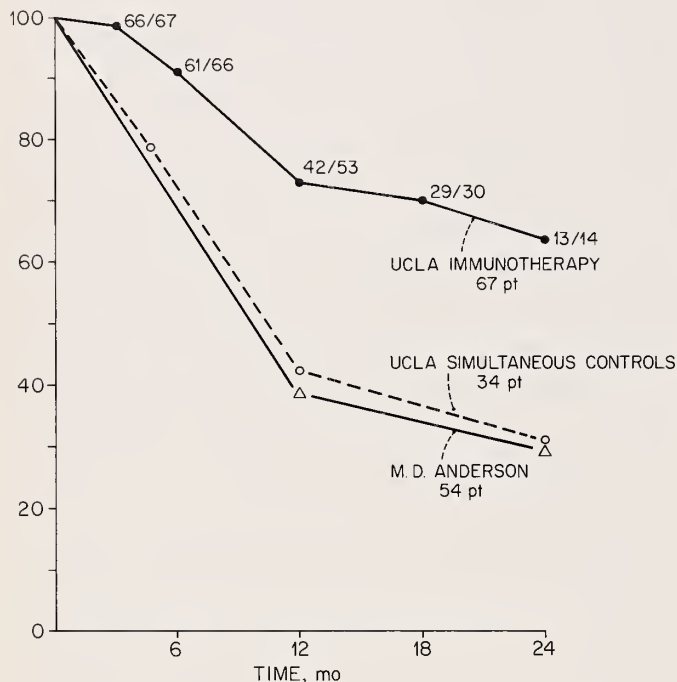
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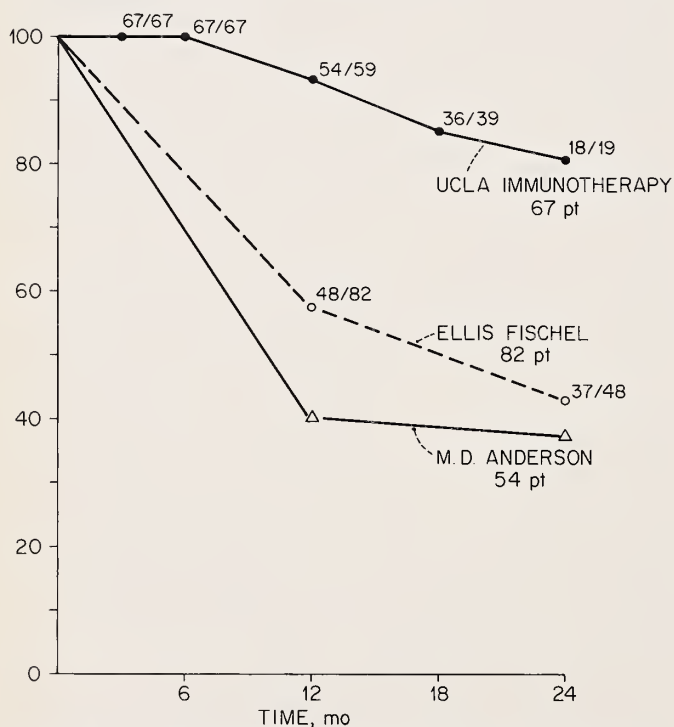
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seen at the University of California at Los Angeles during the same time interval but did not receive immunotherapy, and in comparison with a group of patients reported from the M. D. Anderson Hospital and Tumor Institute in Houston, Texas (text-fig. 1). Similarly, the survival rate of



TEXT-FIGURE 1.—Recurrence rate: Stage II melanoma. Vertical axis represents percentage of patients free of recurrence at each point in time. Number of patients who had recurrence at risk is indicated at each point.



TEXT-FIGURE 2.—Survival rate: Stage II melanoma. Vertical axis represents percentage of patients surviving at each point in time.

patients with Stage II disease treated with immunotherapy is higher on the curve at all points than historical controls in other series (text-fig. 2).

It is apparent that the majority of these patients have been followed for less than 2 years. The preliminary nature of these findings must be stressed, since the follow-up evaluation of the patients is not over a time period sufficient to make a valid statistical statement. Furthermore, in this pilot study we excluded patients who refused immunotherapy and those who lived too far from the hospital to make immunotherapy practical. However, it appears that BCG immunotherapy may be complementary to surgery.

To answer this question definitely, for the last 7 months we have been randomizing patients into a properly stratified and prospective clinical trial, comparing no additional therapy after surgery with postoperative adjuvant immunotherapy using BCG alone or BCG in combination with allogeneic tumor cell vaccine. These patients are being evaluated sequentially with a variety of immunologic techniques in an attempt to develop in vitro assays with which to monitor the response to therapy. These assays include complement-fixation titers to melanoma antigens, immune adherence to melanoma cells, lymphocyte blastogenesis to various mitogens and antigens, mixed lymphocyte cultures, and lymphocyte-mediated cytotoxicity. Sera and lymphocytes are collected sequentially from all patients in the study, and the viable lymphocytes are frozen in aliquots. With this technique, a bank of lymphocytes obtained at various time intervals throughout therapy can be maintained for each patient. At any given time, lymphocyte function and cytotoxicity can be evaluated and compared with the clinical course. We are hopeful that the correlation of the data from these in vitro assays with the clinical course of the disease will be useful in monitoring the response to immunotherapy when the disease is subclinical and cannot be monitored by the standard clinical tests.

We are cautiously optimistic that we will be able better to define the potential of immunotherapy when these prospective randomized studies are completed. However, immunotherapy remains a highly experimental technique; it is already clear that immunotherapy will not cause a dramatic regression of far advanced disease, but it will undoubtedly be most useful against subclinical microscopic amounts of tumor. If, through the integration of laboratory and clinical research, an effective immunotherapeutic adjuvant is developed that can destroy small foci of residual tumor cells, we will at long last have a truly rational basis for radical cancer surgery. Perhaps immunotherapy will eventually find its place as an adjunct to other forms of treatment such as radiation therapy and chemotherapy.

Whatever the future holds in terms of immunotherapy, the methods and techniques of tumor immunology have broad application. Probably, the greatest contributions of the discipline of tumor immunology will be in the areas of diagnosis and etiology.

REFERENCES

- (1) MORTON DL, EILBER FR, HOLMES EC, et al: BCG immunotherapy of malignant melanoma: Summary of a seven-year experience. *Ann Surg* 180:635-643, 1974
- (2) MORTON DL, EILBER FR, MALMGREN RA, et al: Immunologic factors which influence response to immunotherapy in malignant melanoma. *Surgery* 68:158-164, 1970
- (3) SPARKS FC, SILVERSTEIN MJ, HUNT JS, et al: Complications of BCG immunotherapy in patients with cancer. *N Engl J Med* 289:827-830, 1973

Nonspecific Antigen Reactions^{1,2}

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This paper is dedicated to our friend, Dr. Felix Milgrom, State University of New York at Buffalo, on the occasion of the thirtieth anniversary of his research activities.

SUMMARY—Cell-mediated immune challenge reactions to a number of antigens at tumor sites resulted in regressions of various types of benign, premalignant, and malignant lesions in man. Regressions varied from partial to complete and lasted from several months to more than 10 years. Increased levels of cell-mediated immunocompetence were attained by several means, including reduction of tumor burden, immunopotentialization, or transfer of immunity. Antitumor activities of immune challenge reactions are selective for tumor cells and appear to be independent of the nature of the antigens used or the type of neoplasm. Combinations of immunotherapy with other treatment modalities resulted in augmentation of antitumor effects. Preliminary studies indicate that cellular and noncellular mediators of delayed hypersensitivity induce antitumor activities that may be significant in the regressions of neoplasms induced by cell-mediated immune challenge reactions. Since cell-mediated immune reactions are frequent occurrences, they may be a factor in apparently spontaneous regressions of neoplasms.—*Natl Cancer Inst Monogr* 44: 87-97, 1976.

Regressions of neoplastic disease in man and animals, without known therapeutic intervention, have been recognized, although the mechanisms by which such remissions occur are obscure. Recent developments in tumor immunology, particularly in the area of cell-mediated immunity, may provide some explanations for apparently spontaneous regressions of tumors, but a clear relationship has not been established. Results of exploratory immunotherapy for tumors have been variable.

Regressions of accessible lesions following induction of cell-mediated immune challenge reactions at tumor sites have been demonstrated in man and in animals (1-12). These observations have been explored as a basis for extending immunotherapeutic approaches to deep-seated cancers. However, the mechanism of antitumor effects of immune challenge reactions are only partially and inadequately understood. The present status of these studies will be reviewed as they may relate to spontaneous tumor regressions.

Initial clinical studies (1-3) on malignant and premalignant epidermal tumors showed that induction of delayed hypersensitivity reactions to a variety of antigens

at tumor sites was followed by selective eradication of neoplastic lesions. The same approach was subsequently found (4, 5) to induce local regressions of other types of malignant lesions involving the skin and subcutaneous tissues. In the course of these studies, a number of immunologic and nonimmunologic factors were investigated to determine whether and to what extent they may contribute to or reduce the therapeutic effectiveness of immunologic approaches (6-8). The factors under study included chemical antitumor agents, ionizing radiation, and separated components of the immune system.

It has been shown (4-11) that immunotherapy for skin tumors provides an approach for the management of intractable premalignant and malignant epidermal neoplasms. Immunotherapy also can result in reduction of the incidence of new malignant lesions in multiple skin cancer syndromes, which in their natural course are characterized by continuous development of new tumors (3, 12).

Secondary malignant lesions of a wide range of types of cancer involving the skin can also be controlled by immunologic approaches (4, 12). Manipulation of immunologic factors may, therefore, provide a basis for altering the clinical course of malignant tumors.

Elucidation of the actions of components of the immune and other host defense systems has provided some understanding of the mechanisms for controlling the development and the regression of neoplasms (13-21). Our studies (12-14) were primarily concerned with components of the effector systems through which immunologic and other modalities induce regression of malignant lesions. Further understanding of these systems may permit amplification of the underlying mechanisms and utilization of effector agents for improving the control of malignant diseases.

Exploration of general immunopotentialization and other approaches (22-31) to stimulate the immune system for the control of cancer may be guided by understanding the mechanism through which local immunotherapy leads to eradication of malignant lesions. Studies on combining immunologic procedures with other therapeutic modalities have shown that combinations of different treatment methods can result in therapeutic responses when single modalities are not effective. Efforts to extend these principles to the treatment of generalized malignant disease are under investigation.

CLINICAL STUDIES

We observed previously that the development of cell-mediated challenge responses at the sites of neoplastic lesions resulted in regressions (1-8). The initial series of observations (1-3), which was the first demonstration that the immune mechanism can be controlled for the treatment of malignant disease in man or in animals, was done on patients with multiple skin cancer syndromes. Further

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studies demonstrated that analogous principles apply to other types of cancer. Antitumor effects of cell-mediated challenge reactions were induced by a number of different sensitizing agents and resulted in regressions of various types of tumors.

Immunotherapy For Skin Tumors

Initially, immunotherapeutic approaches were explored in patients with primary epidermal neoplasms, which included multiple extensive premalignant epidermal lesions (e.g., lentigines, keratoses, leukoplakia), multiple basal cell carcinomas, and squamous cell carcinomas.

The effects of immune challenge on superficial epidermal tumors have been studied during the past 10 years on more than 125 patients with multiple skin cancers and premalignant lesions. The initial group of patients had intractable skin cancer syndromes that could not be treated adequately by standard modalities. The diseases included multiple basal cell nevoid syndromes, late radiation dermatitis with malignant degeneration, xeroderma pigmentosum, arsenical dermatitis, and severe actinic skin damage due to excessive exposure to ultraviolet light.

In the initial group of 24 patients with multiple epitheliomas, more than 90% of neoplasms were eradicated without residual tumors and without recurrences for observation periods of 7–10 years. A second group of 54 patients with less severe cutaneous carcinomas was followed during the past 5 years with essentially similar results. A subsequent group of 53 patients was followed for the past 2 years by modified protocols that were based on the data obtained from prior exploratory studies. The data obtained so far are in agreement with those obtained in the previous studies. These studies indicate that a course of delayed hypersensitivity challenge reactions results in eradication of epidermal tumors.

Detection of Clinically Unrecognized Neoplasms During Immune Challenge Responses

Immune challenge reveals otherwise undetectable neoplasms by causing inflammatory reactions at the involved sites, which results in resolution of these early tumors. In addition, it is likely that tumors too small to show a grossly recognizable inflammatory reaction are also regressing after immune challenge. Apparently this response, although it cannot be seen clinically, results in resolution of tumors at the earliest stages. This agrees with the findings that courses of immunotherapy in patients with multiple skin cancer syndromes are followed by a decrease in the subsequent incidence of neoplasms. This decrease in incidence is in contrast with the unchanged or higher incidence of tumors in the natural course of, or following other forms of treatments of, multiple epitheliomas.

Decreased Incidence of Skin Tumors Following Immunotherapy

Patients with multiple skin cancer syndromes usually continue to develop tumors throughout the major part of their life. Standard therapeutic procedures (e.g., surgery or radiation therapy) are frequently not satisfactory in view of the large proportion of the body surface involved. Since these modalities are limited to detectable lesions, subsequent tumor development is not reduced.

The incidence of tumors in patients with xeroderma pigmentosum and patients with multiple basal cell nevoid syndromes was markedly reduced following immunotherapy during observation periods of 5–9 years. Studies were

performed on 6 patients at symmetrical anatomical sites: One side received intermittent immunologic challenge while the other side was treated by standard therapeutic modalities. The incidence of new tumors was reduced on the side receiving immunotherapy as compared with the other side during 1-year observation periods. Most lesions occurring after immunotherapy could be managed by subsequent immunologic challenge regimens with or without concurrent topical chemotherapy, since they did not progress to more advanced stages.

Immunotherapy Approaches Based on Immunologic Memory

A number of patients with multiple epidermal tumors, who were unable to develop immune reactions to several primary sensitizing agents, had retained immunologic memory for recall antigens. Apparently they had been exposed to these antigens, which were of microbial origin, prior to impairment of immunocompetence. These antigens included purified protein derivative of tuberculin (PPD), mumps, vaccinia, streptokinase/streptodornase, *Candida* extract, histoplasmin, coccidioidin, blastomycin, *Trichophyton*, pertussis, and other antigens of viral, bacterial, or fungal origin.

Cell-mediated challenge reactions based on immunologic memory were found (12) to provide a basis for inducing selective antitumor effects that resulted in eradication of premalignant and malignant epidermal tumors as well as in eradication of cutaneous lesions of mycosis fungoides, reticulum cell sarcoma, and other nonepidermal tumors. The methods resemble essentially those described previously for eliciting hapten-induced challenge reactions (12). Initially, microbial antigens were administered at varying concentrations by intralesional injections. It was found subsequently that topical administration of microbial antigens was equally or more effective (figs. 1–3).

Immunotherapeutic approaches based on immunologic memory could be extended to patients with extensive malignant disease whose impaired immunocompetence did not permit them to develop de novo cell-mediated immunity. The data also indicate that sensitizing agents of relatively large molecular weight can produce reactions similar to those induced with haptens of small molecular weight. Microbial antigens, particularly PPD, were as effective or more so when compared with dinitrochlorobenzene (DNCB) in regard to the antitumor effects of the respective challenge reactions. Tumor resolution, however, did not occur unless hypersensitivity to the agents used was demonstrated, which indicated that the responses were associated with immunologic processes rather than with other mechanisms. These findings indicate a general relationship of cell-mediated immunity to tumor biology. They further raise the possibility that natural exposure to microbial antigens may result in increased effectiveness of immunologic surveillance.

Immunopotentialiation

Patients with weak or no PPD responses received BCG to determine whether responsiveness to PPD could be increased or induced, respectively. BCG administration, previously explored in patients with advanced disseminated disease (18), resulted in induction or marked increases of responses to PPD as well as to other microbial antigens. These observations further suggest that the immunopotentiating effects of BCG also include an increase in the capacity to develop immunity to primary antigens. Continued administration of BCG, however, was followed by a

decrease in responsiveness to challenge after initial increases in the immune skin reactions (18).

Studies on Mycosis Fungoides

Immunotherapy in mycosis fungoides (MF) has shown marked clinical responses, particularly in patients at early stages. At more advanced stages of MF, regressions were less marked in most patients but were of clinical significance. Topical immunotherapy at the early stages of mycosis fungoides (eczematoid stage, early plaque stage) resulted in marked regression or control of the disease for protracted periods. In 4 of the 7 patients, manifestations of MF were controlled by topical challenge reactions to DNCB, PPD, or *Candida* extract (figs. 4, 5). The other 3 patients showed marked but incomplete regressions following challenge with these agents. Of 9 patients with moderately severe to advanced disease (tumor or ulcerative nodular state, exfoliative erythroderma), 4 patients showed clinically significant responses to immunologic therapy with PPD and DNCB.

Use of topical nitrogen mustard (NH_2) was explored in 8 patients, 3 of whom had previously received topical NH_2 therapy but had had to discontinue it because of the severity of the cutaneous reactions. Since the intensity of the side effects on the skin were due to an allergic reaction, NH_2 was titrated to a concentration at which the normal skin showed minimal or no reaction while the lesions showed considerably more reactivity. Biopsies of normal and neoplastic areas undergoing reactions to NH_2 showed the histologic picture of a delayed hypersensitivity response.

Administration of NH_2 was more satisfactory as an ointment than as a solution, because the concentration of the agent at sites of application could be more adequately controlled. Concentrations ranging from 10 to 0.001 mg of NH_2 per gram of ointment were explored. A concentration adequate to induce a therapeutic response with minimal or no adverse effects of normal tissue was established for each patient. Of 8 patients, 7 tolerated two or more frequent daily applications of NH_2 ointment without significant side effects. Therapeutic responses in 5 of the 8 patients for whom these preparations were studied resulted in sustained, virtually complete control for periods of up to 3 years. Topical NH_2 therapy was generally more effective prior to the development of tumor stage.

Of 7 patients with erythrodermic exfoliative disease involving the entire body surface, 1 patient underwent complete regression following treatment with PPD ointment. This remission has lasted for 30 months (length of observation period up to present). Three patients showed partial responses (1 to PPD ointment, 2 to nitrogen mustard ointment) and 3 others showed no responses to immunotherapy initially. Four of the patients with erythrodermic disease were treated with electron-beam therapy. All improved after whole-body exposure at cumulative doses ranging from 500 to 3,000 rads. One of the patients at the erythrodermic stage, who also had deep-seated disease and involvement of the lymph nodes, responded to combination chemotherapy including prednisone, vincristine, and Cytosan. Following improvement on radiation therapy or chemotherapy, 3 of 4 patients showed significant skin-test responses, which had been minimal or absent initially. Of the 4 patients who had initially failed to respond to immunotherapy, 2 showed partial responses to NH_2 ointment when recurrences took place after X-ray or chemotherapy.

For 3 patients who had ulcerative nodular disease, combinations of radiation therapy with immunologic challenge for individual lesions appeared to be more effective than single modalities. The radiation doses required for control of lesions could be significantly lowered (e.g., 100–250 rads, single dose) in the presence of an immune challenge reaction, as compared with courses of 1,000–3,000 rads in the absence of an immune challenge reaction. These preliminary data indicate that further exploration of combining immunotherapy and radiation and/or chemotherapy may be warranted (figs. 6, 7).

Immunotherapeutic Approaches in Breast Cancer

Studies on the effects of cell-mediated immune challenge reactions on soft tissue lesions of metastatic breast cancer have been initiated (27). Systemic immunopotentialization followed by local immunologic manipulation or other forms of therapy (adrenalectomy, chemotherapy, and hormone therapy) have been explored. Local immunotherapeutic procedures resulted in complete regressions of treated lesions, proved by serial biopsies, in 7 of 14 patients with soft tissue involvement (skin, subcutaneous tissue, and muscle). Regressions (figs. 8–10) lasted for 6 months to more than 1 year. Partial regressions (resolution of 50% of treated lesions, at least a 50% decrease in size of lesions) were observed in 3 patients; there were no detectable responses in 3 others. Regressions were limited to lesions responding to immune challenge.

Three patients in this group of 14 showed no response to skin tests with microbial antigens and could not be induced to respond to DNCB. After these 3 patients had received BCG, they became positive to PPD as well as to other microbial antigens. Two of these patients could then be sensitized to DNCB. Administration of BCG to the other 11 patients resulted in 6 patients showing increases in the intensity of responses to PPD as well as to other microbial antigens to which they had been reacting previously. In addition, administration of BCG was followed by responses to recall antigens to which they had not responded before. After induction of skin-test responses following BCG administration, the agents to which the patients responded were used in local immunotherapy (PPD, mumps, DNCB) with ensuing regressions of challenged lesions.

The period of observation is too short and the number of patients too small to determine whether BCG administration affected the course of the disease. Studies are being continued to determine whether immunopotentialization contributed to therapeutic effects of other forms of therapy (adrenalectomy, chemotherapy, hormone therapy).

Studies on Lentigines

The effects of immunologic challenge reactions to DNCB and PPD were initially explored at sites of lentigines in patients with melanoma and multiple pigmented (benign) nevi. The maintenance of challenge reactions by continuous topical applications of antigens for periods ranging from 12 weeks to several months resulted in gradual loss of pigment and involutions of lesions. Lentigines did not recur at treated sites for observation periods of up to 5 years. Subsequently, nevi and other types of melanin-containing benign lesions, including common freckles, were found to involute during cell-mediated challenge. The involution was selective for pigmented lesions without significant effects on adjacent epidermal or connective tissue elements. Histologic examination showed typical delayed hyper-

sensitivity reactions. It was subsequently found that the concurrent addition of topical 5-fluorouracil to locally administered antigens markedly accelerated the disappearance of pigmented lesions.

Studies on Malignant Melanoma

Local immunologic challenge at tumor sites located in the skin or subcutaneous tissues of patients with metastatic malignant melanoma caused regressions of 30% or more of treated lesions in 4 of 7 patients. There was no evidence of distant therapeutic effects. Local effects of immunologic challenge did not appear to relate to the course of deep-seated metastases. Although regressions of superficial lesions could be induced by challenge with DNCB, PPD, or BCG, deep-seated metastases in the same patients continued to progress.

The effects of BCG, oral or intradermal, on recurrences of melanoma have been studied in 30 patients apparently free of disease after surgery. Evaluable data on clinical effects are not sufficient to permit definitive conclusions. Of 19 patients randomized to BCG or no treatment, who were followed for periods of 6–17 months, 3 developed recurrent disease (2 of 12 patients on BCG). The overall effect of immunopotentialization with BCG was an increase in skin-test responses both in regard to number of antigens eliciting reactions and in the intensity of the reactions. Temporary reductions or complete suppression of skin-test responses occurred in 6 of 12 patients receiving BCG. Significant changes in skin-test responses failed to occur in patients who were not receiving BCG.

We are currently comparing the effects of a combination of three chemotherapeutic agents [5-(3,3-dimethyl 1-triazeno)-imidazole-4-carboxamide, vincristine, and 1,3-bis(2-chloroethyl)-1-nitrosourea] and BCG (intradermal) with the effects of chemotherapy without BCG in 27 patients with unresectable metastatic melanoma. The number of partial and complete regressions in the groups receiving chemotherapy in combination with BCG exceeds that in the groups receiving chemotherapy alone. The statistical and clinical significance of these findings require further evaluation.

Studies on Separated Components of the Immune System

Preliminary explorations of the effect of intralesional administration of leukocytes and noncellular mediators of delayed hypersensitivity reactions have been performed (18). Intratumoral administration of lymphokine preparations resulted in partial to complete regressions of cutaneous lesions of basal cell carcinomas (2 of 3 patients), adenocarcinoma of the breast (2 of 4 patients), and infiltrates of mycosis fungoides (3 of 5 patients). Intralesional administration of concentrates of human monocytes (5×10^7 cells/ml) resulted in temporary and partial regressions (18) of cutaneous lesions of breast cancer (2 of 3 patients), reticulum cell sarcoma (2 of 3 patients), and squamous cell carcinoma (1 patient) (figs. 7, 8). Intralesional administration of concentrates of peripheral human leukocytes or lymphocytes had no direct effects on neoplastic lesions (6 patients) but transferred immunity to haptens and microbial antigens in 18 of 26 patients who had been initially unable to develop cutaneous challenge responses to the same sensitizing agents. The immunity transferred through intact leukocytes or transfer factor (4, 29) could then be used to induce antitumor effects by challenge reactions.

The observations that tumors regressed following administration of components of the cell-mediated immune

system are consistent with the antitumor effects of delayed hypersensitivity challenge reactions. They illustrate one type of mechanism that may account for the findings reported here. The observations that monocytes, not activated by lymphokines, can also exercise antitumor effects in vivo as well as in vitro indicate the presence of a recognition mechanism not dependent on the immune system that permits selective action of monocytes against tumors in a manner similar to that exercised against microorganisms. These in vivo observations in human tumors are in agreement with in vitro studies by several authors (12, 14–17).

DISCUSSION

Regressions of tumors following immune reactions or other inflammatory processes have been recognized, and some aspects of possibly related phenomena have been described here. As demonstrated in previous studies, tumors involving the skin provide considerable investigative flexibility as compared with deep-seated tumors. Their location facilitated the observation of phenomena not readily recognized when they occur in deep-seated organs; their accessibility further permitted the recognition of selective immune antitumor activity, which could then be extended to a wide spectrum of tumors. Immunotherapeutic approaches explored in epidermal tumors could therefore be applied to metastatic lesions of disseminated malignant diseases involving the skin. It is possible that apparently spontaneous regressions of tumors may have been observed, which were related to factors described here but were not recognized prior to the recent understandings of some mechanisms of host defense systems.

Our studies revealed findings of high 5-year cure rates following immunotherapy of primary epidermal tumors, early recognition of clinically undetectable lesions, and reduced incidence of new malignant lesions in skin cancer syndromes that usually show persistent development of new tumors.

These findings appear to be significant for immunotherapeutic approaches to other types of cancer. Regressions of metastatic lesions of melanoma and adenocarcinoma of the breast involving the skin and soft tissues and of disseminated lesions of cutaneous lymphoma indicate the need for a systematic investigation to determine the mechanisms by which immunologic approaches may be developed for the treatment of these and other neoplastic diseases at earlier stages.

Selective eradication of premalignant lesions by immunologic procedures prior to progression to the frankly malignant state may be a form of surveillance by control of the transformation of cells at a stage at which they develop early antigenic changes. Furthermore, immunoprophylaxis may be feasible at premalignant states, at which morphologic or biochemical characteristics of neoplastic changes cannot be recognized. Studies on immunoprophylaxis may, therefore, be warranted in syndromes that are associated with a high incidence of malignant disease. In multifocal cutaneous cancer syndromes in which progression of the disease usually results from de novo neoplasia rather than by dissemination of already present malignant cells, immunotherapy contributes to the control of established as well as subsequent neoplastic lesions.

Since immunotherapeutic approaches pertain to a spectrum of neoplastic diseases in man, it appears warranted to explore the antitumor effects of cell-mediated challenge re-

actions on tumors that have not yet been investigated in this respect. We have investigated malignant lesions in the genitourinary tract, in collaboration with Merrin et al. (12, 30). Similar approaches are feasible for tumors in the head and neck region, the intestine, and other directly or indirectly accessible anatomic sites. The results of immunologic procedures in disseminated malignant lesions indicate that palliative effects may be obtained when standard methods of treatment have failed. Furthermore, serious toxicity or generalized anaphylactic reactions have been reported but have not been encountered by us in more than 300 patients under study for periods up to 10 years. On this basis, further studies on immunotherapeutic approaches to intractable disseminated malignant disease appear warranted.

Antitumor effects of immunologic challenge reactions based on immunologic memory may be of theoretical as well as practical significance. From a practical point of view, immunologic memory permits immunotherapeutic approaches in patients whose immunocompetence had been impaired in respect to developing immunity to primary antigens. We have found that this impairment frequently occurs before loss of immunologic memory. Antitumor effects are essentially similar, whether small organic chemical agents (haptens) or microbial antigens of larger molecular weight are used to elicit challenge reactions.

Since two or more sensitizing agents used concurrently produce more intense antitumor effects than single agents, it appears that combinations of antigens have mutually enhancing or coantigenic effects. The concurrent administration of two or more antigens produces delayed hypersensitivity responses at concentrations markedly below those at which the same antigens as single agents fail to elicit challenge reactions. Similar considerations may pertain to eliciting a response to otherwise weak tumor antigens. The presence of one or more unrelated antigens may initiate a response that then stimulates or induces reactions against tumor antigens. Such a coantigenic effect may be responsible for more intense challenge reactions at tumor sites than in normal tissues. The coantigenic effects following concurrent administration of two or more sensitizing agents may be of further significance in respect to antitumor action of microbial vaccines such as BCG, which contain a number of antigenic determinants.

Combinations of local chemotherapy and immunotherapy were more effective in controlling tumors than were single modalities (4, 12, 27). Antitumor effects could further be increased when chemotherapeutic agents were combined with more than one immunologically active agent. Initially, topical chemotherapy was combined with an immune challenge reaction. Paradoxically, rather than inhibit the immune reaction, the intensity of the response to the combination considerably exceeded the reaction produced by each modality alone, and without loss of selectivity for tumor destruction. Systemic chemotherapy combined with local immunotherapy was subsequently explored. Agents initially used systemically without immunosuppressant effects included bleomycin and triacetylazauridine. Indications of additive antitumor effects were obtained in patients with mycosis fungoides, reticulum cell sarcoma, and squamous cell carcinoma when systemic chemotherapy was combined with local immune challenge. Recently initiated studies are using vinblastine, Cytosan, or pulsed regimens of high doses of methotrexate followed by citrovorum rescue, as described by Djerassi et al. (31), which permit the re-

covery of immunocompetence between successive chemotherapeutic treatments.

Tumor immunology provides a challenging area for investigation of the complexities of delicately balanced immunologic systems and has stimulated progress in fundamental aspects of immunology, such as the biology of mononuclear cells, the role of noncellular mediators, and their regulatory mechanisms.

Further studies in this area may be important to tumor immunology and also relevant to infectious diseases and other normal and pathologic processes in which immune mechanisms are significant.

Selective destruction of tumor cells associated with delayed hypersensitivity challenge reactions may involve both cellular and noncellular factors. Since tumor regression is observed in the absence of discernible effects on the surrounding normal tissues or the healing process, these factors must possess some degree of selectivity or specificity. Besides immunologically specific factors, nonspecific ones including the various noncellular mediators and cellular elements of delayed hypersensitivity appear to be involved. To what extent these factors possess selectivity is only partially understood. In addition to recognized immune phenomena, other discriminatory mechanisms appear to be significant (12, 14, 32).

Delayed hypersensitivity reactions are associated with local accumulation of mononuclear cells and increased capillary permeability leading to extravasation of plasma proteins. Both cellular and noncellular components of the delayed hypersensitivity reaction seem to be involved in the destruction of tumor cells. Monocytes and macrophages act as effectors of tumor cell destruction, the selectivity for which may be mediated by antibodies cytophilic for the monocytes or the macrophages or by antibodies interacting with tumor-specific antigens on tumor cells, which thus earmark the latter for interaction with the monocytes and macrophages. Noncellular mediators amplify the selective activity of monocytes for tumor cell destruction.

Preliminary experimental observations in our laboratory support this view (14, 32). Peritoneal macrophages from rats obtained after BCG sensitization and PPD stimulation will destroy a number of different syngeneic rat tumors *in vitro*. Serum components from tumor-bearing animals have been found to influence profoundly this *in vitro* interaction between macrophages and tumor cells (13, 14). Thus we have evidence that macrophages can act as effector cells in tumor cell destruction and, furthermore, that serum factors (presumably antibodies) can influence the interaction between tumor cells and macrophages.

Intralesional administration of concentrated human monocytes and lymphokine preparations obtained from human lymphocytes resulted in at least partial regression of tumors in man. These observations appear to be consistent with the findings in animals and in cell culture systems (14, 32).

While immunotherapeutic approaches to several types of malignant disease appear feasible, further pursuit of the reasons for failures of this approach should provide meaningful information. Also indicated is intensive study of the relatively small proportions of patients in whom, so far, immunotherapeutic approaches have exerted clinically significant antitumor effects.

The relationship between a tumor and its host appears to involve a complex array of factors contributing to a delicate balance that may favor either tumor growth or tumor resistance. Both immunologic and nonimmunologic com-

ponents appear to be involved. Immunologic intervention of the type we have described may be effective insofar as it shifts the balance in favor of tumor resistance, but we must also be aware of the possibility of shifting the balance in the wrong direction. Left to its own devices, a tumor may by its nature progressively shift the balance in its own favor. On the other hand, the observation of occasional spontaneous regression may be the consequence of a fortunate natural shift of the balance in favor of the host.

REFERENCES

- (1) KLEIN E, HELM F: Effects of delayed hypersensitivity reactions on cutaneous neoplasms. Presented at the Stephen Rothman Research Meeting, American Academy of Dermatology, Chicago, Ill., December 1963
- (2) HELM F, KLEIN E: Effects of allergic contact dermatitis on multiple superficial basal cell carcinomas. *Arch Dermatol* 91:142-144, 1965
- (3) KLEIN E: Differential immunologic reactions in normal skin and epidermal neoplasms. *Fed Proc* 26:430, 1967
- (4) —: Tumors of the skin. X. Immunotherapy of cutaneous and mucosal neoplasms. *NY State J Med* 68:900-911, 1968
- (5) —: Local cytostatic chemotherapy and immunotherapy. *Geriatrics* 23:154-175, 1968
- (6) —: Hypersensitivity reactions at tumor sites. *Cancer Res* 29:2351-2362, 1969
- (7) WILLIAMS AC, KLEIN E: Experiences with local chemotherapy and immunotherapy in premalignant and malignant skin lesions. *Cancer* 25:450-462, 1970
- (8) KLEIN E: Local chemotherapy of cutaneous neoplasms. In *Proceedings of the Sixth National Cancer Conference* (Clark RL, ed.). Philadelphia, Lippincott, 1970, pp 201-515
- (9) ZBAR B, BERNSTEIN I, TANAKA T, et al: Tumor immunity produced by the intradermal inoculation of living tumor cells and living *Mycobacterium bovis* (strain BCG). *Science* 170:1217, 1970
- (10) STJERNSWÄRD J, LEVIN A: Delayed hypersensitivity-induced regression of human neoplasms. *Cancer* 28:628-640, 1971
- (11) LEVIS W, KRAEMER K, KLINGER W, et al: Topical immunotherapy of basal cell carcinomas with dinitrochlorobenzene. *Cancer Res* 33:3036-3042, 1973
- (12) KLEIN E, HOLTERMANN OA: Immunotherapeutic approaches to the management of neoplasms. *Natl Cancer Inst Monogr* 35:379-402, 1972
- (13) HOLTERMANN OA, CASALE GP, KLEIN E: Tumor cell destruction by macrophages. *J Med* 3:305-309, 1972
- (14) HOLTERMANN OA, KLEIN E, CASALE GP: Selective cytotoxicity of peritoneal leukocytes for neoplastic cells. *Cell Immunol* 9:339-352, 1973
- (15) ZBAR B, BERNSTEIN ID, RAPP HJ: Suppression of tumor growth by peritoneal exudate macrophages from unimmunized Strain-2 guinea pigs. *Proc Am Assoc Cancer Res* 11:87, 1970
- (16) HIBBS JB, LAMBERT LH, REMINGTON JS: Control of carcinogenesis: A possible role for the activated macrophage. *Science* 177:998-1000, 1972
- (17) ALEXANDER P: Activated macrophages and the antitumor action of BCG. *Natl Cancer Inst Monogr* 39:127-133, 1973
- (18) KLEIN E, HOLTERMANN OA, CASE RW, et al: Responses of neoplasms to local immunotherapy. *Am J Clin Pathol* 62:281-289, 1974
- (19) HOLTERMANN OA, PAPERMASTER BW, ROSNER D, et al: Regression of cutaneous neoplasms following delayed-type hypersensitivity challenge reactions to microbial antigens or lymphokines. *J Med* 6:157, 1975
- (20) MATHÉ G, WEINER R, POUILLART P, et al: BCG in cancer immunotherapy: Experimental and clinical trials of its use in treatment of leukemia minimal and/or residual disease. *Natl Cancer Inst Monogr* 39:165-175, 1973
- (21) LEVENTHAL BG, LÉPOURHET A, HALTERMAN RH, et al: Immunotherapy in previously treated acute lymphatic leukemia. *Natl Cancer Inst Monogr* 39:177-187, 1973
- (22) SOKAL J, AUNGST CW, GRACE JT JR: Immunotherapy of chronic myelocytic leukemia. *Natl Cancer Inst Monogr* 39:195-198, 1973
- (23) GUTTERMAN J, MAVLIGHT G, MCBRIDE C, et al: Immunoprophylaxis of malignant melanoma with systemic BCG: Study of strain, dose, and schedule. *Natl Cancer Inst Monogr* 39:205-212, 1973
- (24) BORNSTEIN BS, MASTRANGELO MJ, SULIT H, et al: Immunotherapy of melanoma with intralosomal BCG. *Natl Cancer Inst Monogr* 39:213-220, 1973
- (25) PINSKY CM, HIRSHAUT Y, OETTGEN HF: Treatment of malignant melanoma by intratumoral injection of BCG. *Natl Cancer Inst Monogr* 39:225-228, 1973
- (26) POWLES R: Immunotherapy of acute myelogenous leukemia in man. *Natl Cancer Inst Monogr* 39:243-247, 1973
- (27) KLEIN E, HOLTERMANN OA, PAPERMASTER BW, et al: Immunologic approaches to various types of cancer with the use of BCG and purified protein derivatives. *Natl Cancer Inst Monogr* 39:229-240, 1973
- (28) MORTON DL, EILBER FL, MALMGREN RA, et al: Immunologic factors which influence response to immunotherapy in malignant melanoma. *Surgery* 68:158-164, 1970
- (29) LAWRENCE HS: Transfer factor. *Adv Immunol* 11:195-266, 1969
- (30) MERRIN C, HAN T, KLEIN E, et al: Immunotherapy of prostatic cancer with bacillus Calmette-Guérin and purified protein derivative. Preliminary Results. *Urology* 2:651-654, 1973
- (31) DJERASSI I, ROMINGER CD, KIM JS, et al: Phase I study of high doses of methotrexate with citrovorum factor in patients with lung cancer. *Cancer* 30:22-30, 1972
- (32) HOLTERMANN OA, DJERASSI I, LISAFELD BA, et al: In vitro destruction of tumor cells by human monocytes. *Proc Soc Exp Biol Med* 147:456-459, 1974



FIGURE 1.—Patient with multiple basal cell carcinomas prior to immunotherapy.

FIGURE 2.—Same patient as shown in figure 1, responding to challenge with topical PPD (preparation 10,000 IU PPD/g aqueous base).

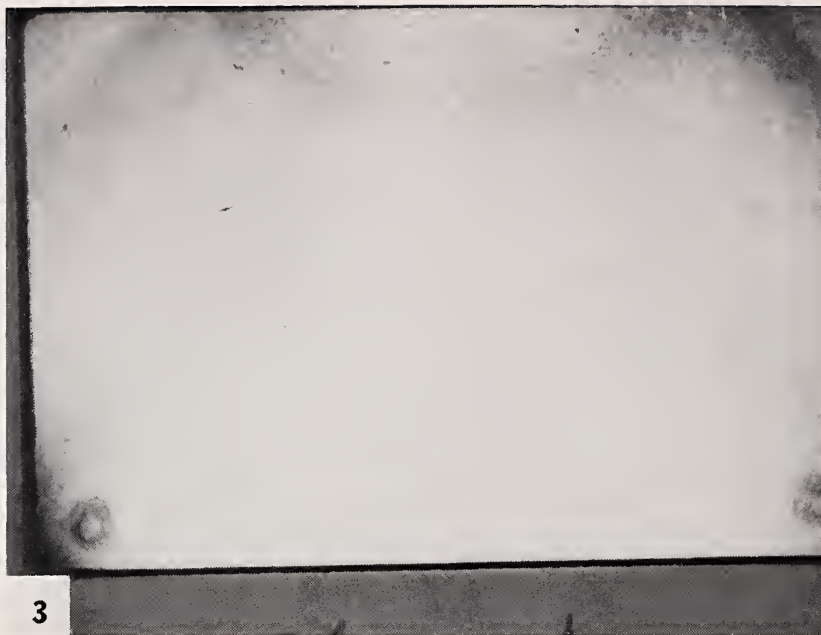


FIGURE 3.—Same patient as shown in figures 1 and 2 at 3 weeks after terminating a 4-week course of daily PPD administration. More than 90% of tumors have been eradicated.

FIGURE 4.—Lesions of mycosis fungoides on forearm.

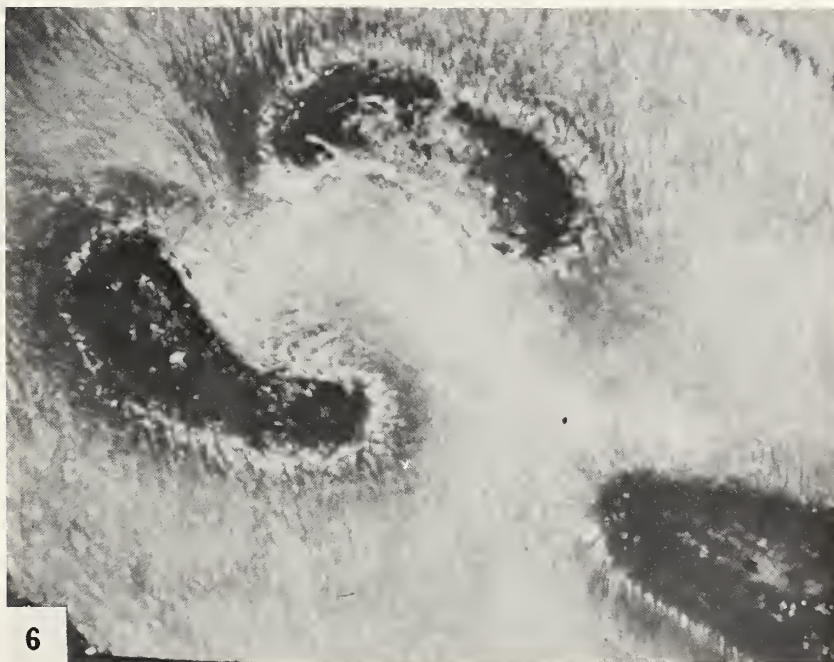


FIGURE 5.—Same patient as shown in figure 4 after 3-week course of daily topical administration of PPD (100,000 IU PPD/g aqueous base). Complete clinical regression confirmed by microscopic examinations lasted for duration of observation period (12 mo).

FIGURE 6.—Lesions of mycosis fungoides that have failed to respond to single treatment modalities (X-ray, chemotherapy, immunotherapy).

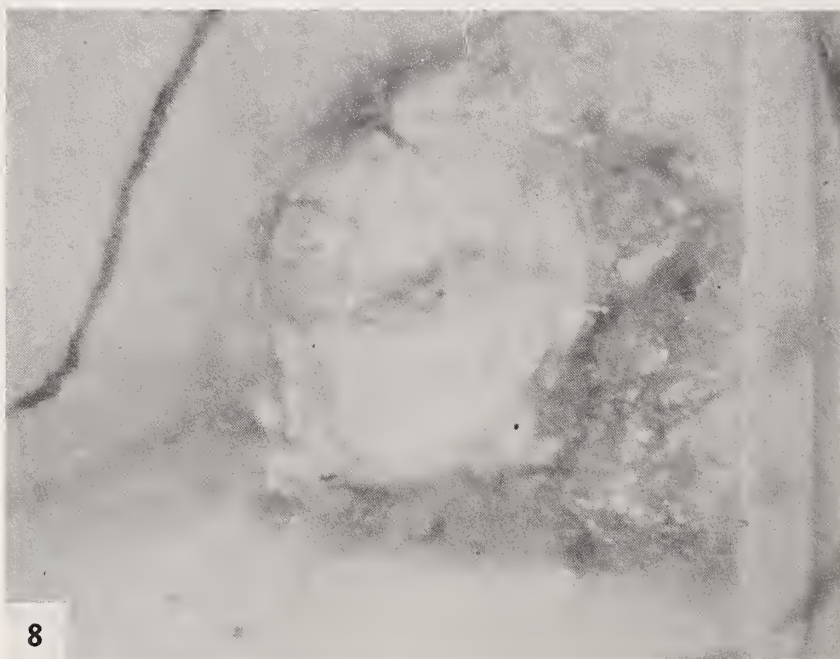
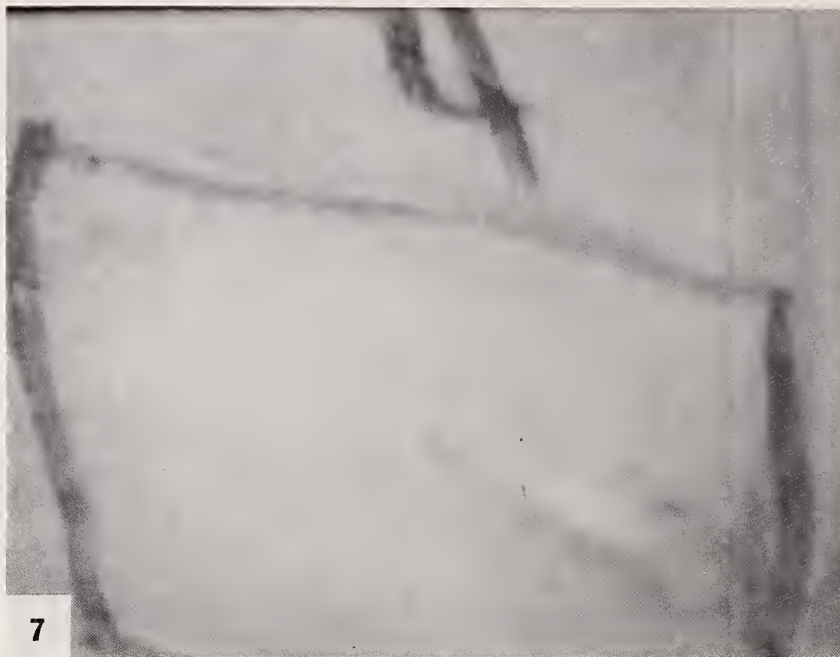


FIGURE 7.—Same area as shown in figure 6 after combining X-ray therapy (500 rads/single dose) with concurrent cell-mediated challenge. Regression of lesion lasted for observation period of 36 mo.

FIGURE 8.—Patient with soft tissue recurrences of breast cancer prior to immune challenge regimen.

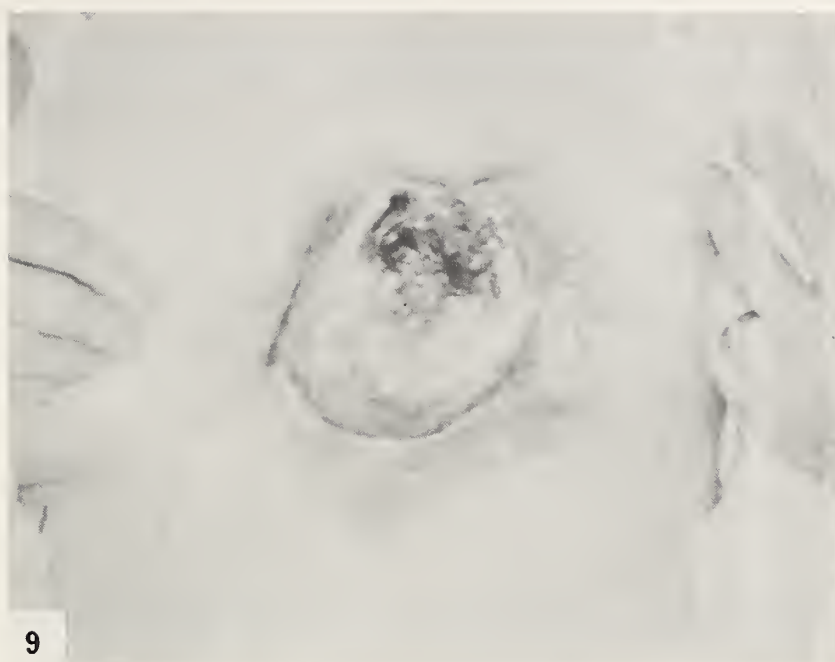


FIGURE 9.—Same area as shown in figure 8. Partial regression has occurred following course of cell-mediated challenge to topical PPD.

FIGURE 10.—Same patient as shown in figures 8 and 9 after combination of topical chemotherapy (5-fluorouracil) and immune challenge courses. Regression lasted for observation period of 14 mo.

Latent Carcinoma and Carcinoma In Situ¹

G. Barry Pierce and Robert H. Fennell, Jr.²

SUMMARY—Lesions exist in the cervix that are diagnosed as carcinoma in situ; some may progress to invasive carcinoma and some may regress. Many are probably overdiagnosed, may represent effects of promoting agents rather than initiating agents, and may entail risk for the patient in that the lesion may be an unusually good target in carcinogenesis. Latent carcinomas are small foci of initiated cells unable to express their malignant phenotype because of environmental controls. Latent cells are produced in experimental carcinogenesis and occur in spontaneous tumors as G-O stem cells.—*Natl Cancer Inst Monogr* 44: 99-102, 1976.

The tenor of this meeting thus far has established the notion that spontaneous regression of established malignant tumors can occur, although so rarely that it is of no clinical significance and offers no hope for patients with cancer. Through study of these rare cases, it was hoped to develop insights that ultimately might be exploited therapeutically. The prevailing thread in the papers has been the idea that there is probably a successful reaction of the immune system with rejection of the tumor. Dr. Evans introduced the notion that for neuroblastoma, abrogation of malignancy might come about through differentiation of neuroblastoma cells to ganglioneuroma cells (1). We will expand upon this notion.

The subjects assigned to us for consideration are well-described clinical entities, although neither is well understood. From a morphologic standpoint, latent carcinoma and carcinoma in situ are considered to be malignant tumors, although not expressing the capacity for invasion and metastasis characteristic of most cancers. Peterson (2) and Peterson and Wiklund (3) suggested that these entities are stages in the development of invasive metastasizing carcinomas, since 39 of 127 cases that they observed eventually progressed to invasive carcinomas. Rather than implicate immunity as the primary control phenomenon, aspects of cell and developmental biology will be proposed. The data are fragmentary, and more problems will be identified than will be solved.

Carcinoma in situ will be considered only in the uterine cervix because it has been subjected to more study in that situation than in any other (2-8). The cells of a carcinoma in situ have the cytologic features of malignant cells with the single exception that there is no evidence of invasion (fig. 1). It is to be stressed that the morphologic alterations are not minimal in degree, rather they are the equivalent of about a Broder's Grade III carcinoma confined to the epithelium and separated from the stroma by an intact basement membrane. There is some evidence that these lesions when left untreated eventuate in invasive carcinoma, but the incidence and time of this progression are not

known. Indeed, there are those who question the significance of carcinoma in situ as a precursor of invasive cancer. In a group of 576 patients with carcinoma in situ, 75 had persistent disease after initial therapy; these were followed for 1-11 years without the development of invasive cancer (7). Dr. Firminger (9) has cited Erickson's observations that many lesions diagnosed as carcinoma in situ do not become invasive. If biopsy cures these lesions, one must assume regression in at least some of them.

Occasionally, one may see downgrowths of squamous epithelium from what is otherwise carcinoma in situ (2, 5, 8) (fig. 2), or from what appears to be more mature epithelium not qualifying as carcinoma in situ (fig. 3). These buds are often more differentiated than the cells of carcinoma in situ and may be in vascular channels (fig. 4). Our interpretation is that these represent the earliest stage of invasive carcinoma, although Glucksmann and Cherry question the idea (5). Although very few of these lesions, which we call microinvasive carcinoma, have metastasized (8), the Stockholm experience (6) indicates that it, not carcinoma in situ alone, develops into invasive carcinoma.

Thus we are left with an interesting contradiction: On the one hand is the highly malignant-appearing carcinoma in situ that lacks invasive properties, and on the other hand is the less malignant-appearing tumor with the capacity to invade and presumably develop as an invasive squamous cell carcinoma. Some believe the latter to be derived from the former; at least they occur in association.

As an alternative to the notion that one is derived from the other, it is now well known that carcinogenic events involve many normal stem cells; the state of differentiation of the targeted stem cells and their microenvironment probably determine the type and histologic grade of tumor and when it will develop (10). Thus we would postulate that the target cell for carcinoma in situ is a basal cell, and the target for the microinvasive squamous cell carcinoma is a well-differentiated squamous cell still capable of synthesizing DNA. When the skin of an experimental animal is painted with carcinogen, multiple tumors arise from different stem cells and appear almost simultaneously (11). Thus carcinoma in situ and microinvasive carcinoma need not be derived one from the other.

It is interesting that carcinomas in situ have not been observed as a stage in experimental carcinogenesis in mice (12). This does not mean that it is not, therefore, a stage in carcinogenesis; rather, the experimental models developed to study the early stages of malignancy are not necessarily an accurate reflection of all aspects of tumor formation in man. Chemical carcinogens employed in experimental situations are usually noted for their potency and have been highly selected to shorten the bothersome latent period, i.e., the time between application of carcinogen and development of clinically evident tumors. Carcinomas in situ and experimental tumors produced by the painting of rabbit skin with chemical carcinogen have in common that the processes are not smoothly progressive. When the skin of the rabbit was repeatedly painted with

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chemical carcinogen, warts developed; when the painting was discontinued, the warts regressed (11). Thus it was learned that the development of tumors is responsive to environmental conditions. By marking warts with India ink tattoos, it was possible to demonstrate that after regression, when the carcinogen was reapplied, the warts reappeared at the site of tattooing. This happened even after prolonged cessation of painting, which indicates that the initial changes in tumorigenesis are apparently long-lasting ones.

This cyclic behavior of early carcinomas is also found in virus-induced tumors. Shortly after birth, suckling mice acquire mammary tumor virus from the milk of the mother. They are apparently disease free until about 40 weeks of age, which is a considerable time in the life-span of a mouse. At that time, tumors develop and grow rapidly during pregnancy but regress or even disappear between pregnancies. These typical cycles of growth and regression continue until a noncyclic, progressive pattern of growth becomes established (13). This pattern of growth might begin during a long latent period between pregnancies or during pregnancy. Thus virally and chemically induced tumors behave similarly in their early mode of growth; each has a long latent period and the early growth is not smoothly progressive. This also suggests that, contrary to usual dogma, tumors, at least in their early stages, are responsive to growth control. It is not surprising, then, that carcinomas *in situ* might regress. One might anticipate that after regression any initiated cells might be reactivated by appropriate environmental factors. In experimental carcinogenesis, the activating environmental factors have been called promoting agents (14), and the one most commonly used is a phorbol ester derived from Croton oil. The nature of promoting agents in carcinoma *in situ* has never been established.

Raick (15) has conducted an intriguing series of experiments on the effect of phorbol esters on normal skin and on skin that had been previously painted with an amount of carcinogen that by itself would not cause tumors. He studied and compared the early ultrastructural changes in the normal and initiated skin treated at weekly intervals with the promoting substance. Noninitiated and initiated skin reacted identically. The cells underwent a series of phenotypic changes that, at best, could be described as dysplastic. Some of these cells were indistinguishable from malignant stem cells, an observation which raises several interesting possibilities. The first would be that carcinogen initiates cells in the cervix, which then respond to environmental phenomena and progress or regress depending upon alterations in the environment. The other possibility is that the cervix may be bathed by promoting substances that may cause changes in the phenotypic appearance of cells not initiated and not yet malignant. Thus it is conceivable that what we are observing in the cervix and diagnosing as carcinoma *in situ* may not contain malignant cells. If this is true, it is conceivable that the association of invasive squamous cell carcinoma in patients who have had carcinoma *in situ* may be the propensity for these hyperplastic or dysplastic lesions to undergo carcinogenesis. We favor neither idea, and the dilemma remains.

Latent carcinomas of the prostate have been extensively studied by Moore (16) who observed minute foci of malignant-appearing cells in the prostates of men past the age of 40. These foci were indistinguishable from those of metastatic adenocarcinoma of the prostate taken from either lymph node or osseous metastases. Moore observed an increased incidence of latent carcinomas of the prostate with age, which correlated directly with the increased

incidence of adenocarcinoma of the prostate with age; he came to the conclusion that latent tumors were a stage in the development of clinical adenocarcinoma.

Latency as a phenomenon of carcinogenesis, either chemical or viral, has already been alluded to and has been the bane of molecular biologists investigating this process. It is impossible at the molecular level to study biologic events that develop over months. A phenomenon similar to latency is the clinical phenomenon of dormancy. In this situation, a woman presents with an adenocarcinoma of the breast which is removed surgically, and the patient is apparently cured. She returns for annual check-ups, and many years later presents with a small pea-sized nodule in the mastectomy scar. Histologically, the tumor is the same as the one removed years previously, and the patient usually succumbs quickly to widespread dissemination of tumor. Dormancy differs from latency only in that we know that dormant cells were derived from highly aggressive clinical cancers. Apparently a small number of these cells were sequestered in the mastectomy scar, an environment that proved incompatible with the expression of the malignant phenotype. In other words, there are normally occurring situations in which the malignant phenotype can be effectively controlled. This is an interesting thought because cancer dogma says neoplastic growth is uncontrolled.

Since the phenomena of latency and dormancy involve small numbers of cells, one can ask whether or not there are critical numbers of cells essential for the expression of a particular phenotype. Grobstein and Zwillig (17) studied this problem years ago in the explanted chick embryo. These embryos had been incubated for 24 hours, at which time the head primordium was dissected and cultured. It formed a head in the *in vitro* environment. When this primordium was divided in half and each half was cultured separately, each formed a head. Quarters of the primordium each formed a head, but not all of the eighths did. None of the sixteenths of the head primordium, when cultured separately, formed a head. However, if the sixteenths were aggregated and cultured, they formed a head. The authors concluded that a critical number of cells was required for a morphogenetic movement to take place. It is known that there are critical numbers of cells required to successfully transplant certain tumors and that in many *in vitro* situations, critical numbers of cells are required to establish cultures.

The best experimental model that I am aware of for the study of latency and dormancy is that described by the Fisher brothers (18), one a pathologist and the other a surgeon, who studied the development of experimental liver metastases of Walker carcinosarcoma cells injected into the portal circulation of rats. When 250,000 Walker cells were injected, the animals succumbed in a matter of 3 weeks with massive hepatic metastases. When rats were injected intraperitoneally with 50 Walker carcinosarcoma cells and then examined 5 months later for hepatic tumor, no tumors were present. When animals underwent laparotomy 3 months after injection of 50 tumor cells and laparotomy was repeated weekly thereafter, all of the animals died within a few weeks. Some animals survived for as long as 20 weeks, which indicated that these highly malignant tumor cells lay dormant in the livers until activated by some nonspecific environmental stimulus associated with surgical trauma or wound healing.

Leighton (19) has studied a phenomenon of cell culture that he refers to as aggregate replication. Apparently tumor cells grow together to form small clumps, and the biologic

behavior of these cells then is their aggregated performance in the clump. Thus it is supposed that malignant cells create a microenvironment optimal for their own function and growth, as described for normal cells by Grobstein and Zwilling (17). In the case of dormant tumor cells, too few cells are present to create an optimal microenvironment with the expression of the malignant phenotype, and the malignant cells are controlled by their environment. Many of the progeny of malignant stem cells undergo differentiation to benign cells, thereby tending to reduce the numbers of malignant stem cells (20). It is only when enough stem cells become aggregated and create their own unique environment that they are able to multiply optimally, invade and metastasize, and destroy their hosts.

The controls operative in these situations are not known. In the case of adenocarcinoma of the breasts of mice, presumably endocrinologic types of control operate, and these are augmented by a more intimate type of intrinsic control probably mediated by cell-to-cell interactions. Cell-to-cell interactions have been studied by Moscona (21) and Garber and Moscona (22), who have disaggregated embryonic cells and studied their social requirements in reaggregation. Obviously, much more work is required to pinpoint how this type of control operates, but its importance cannot be overstressed.

It is proposed, then, that latent tumors are ones in which the cells are responding to either intimate or some other type of environmental control, which precludes the expression of the overall malignant phenotype.

As clinicians, we are aware that there are noncycling cells in tumors, which are believed to be resistant to lethal effects of X-rays and to radiomimetic drugs. I would propose that these are really latent tumor cells responding to some type of environmental control that precludes their division. Mendelsohn (23) observed that about 50% of the cells of spontaneous adenocarcinoma of the breasts of mice did not synthesize DNA during a 5-day interval of time and postulated that these cells were arrested in G-O. Since we (24) had previously demonstrated in a squamous cell carcinoma and in teratocarcinomas that highly malignant stem cells could differentiate to benign postmitotic senescent progeny, we were interested in knowing whether nondividing cells of breast carcinomas were stem cells arrested in G-O or were postmitotic as a result of differentiation. Consequently, animals bearing spontaneous adenocarcinomas of the breast were given perfusions of tritium-labeled thymidine for 5 days, and the tumors were examined autoradiographically with light and electron microscopes. About half of the cells did not synthesize DNA during the 5-day interval, and electron microscope autoradiography of the cells not synthesizing DNA showed that 11, 14, and 35% of them were postmitotic because of differentiation. Thus the majority of the nondividing cells were stem cells arrested in G-O and were, for all intents and purposes, latent; that is, they were not in cycle because of some environmental stimulus.

The importance of this phenomenon cannot be overemphasized. As many as one third of the cells of these adenocarcinomas of the breast were not synthesizing DNA

because of some environmental circumstances, which, if we understood them, would provide an alternative to the usual types of cytotoxic chemotherapy.

REFERENCES

- (1) EVANS AE, GERSON J, SCHNAUFER L: Spontaneous regression of neuroblastoma. *Natl Cancer Inst Monogr* 44:49-54, 1976
- (2) PETERSON O: Spontaneous course of cervical precancerous conditions. *Am J Obstet Gynecol* 72:1063-1071, 1956
- (3) PETERSON O, WIKLUND E: Further studies on the spontaneous course of cervical precancerous conditions. *Acta Radiol* 188:210-215, 1959
- (4) FENNEL RH: Carcinoma in situ of the cervix with early invasive changes. *Cancer* 8:302-309, 1955
- (5) GLUCKSMANN A, CHERRY CP: Microinvasive carcinoma of the cervix: Histopathological aspects. In *Dysplasia, Carcinoma In Situ* (Gray LA, ed.). Springfield, Ill., Charles C Thomas, 1964, pp 351-378
- (6) RUBIO C, SODERBERG G: Cytologic, histologic and follow-up studies in 531 cases of carcinoma in situ of the uterine cervix. *J Reprod Med* 3:65-69, 1969
- (7) GREEN GH, DONOVAN JW: The natural history of cervical carcinoma in situ. *Br J Obstet Gynaecol* 77:1-9, 1970
- (8) CHRISTOPHERSON WM, PARKER JE: Micro-invasive carcinoma of the uterine cervix. A clinicopathologic study. *Cancer* 17:1123-1131, 1964
- (9) FIRMINGER H: A pathologist looks at spontaneous regression. *Natl Cancer Inst Monogr* 44:15-18, 1976
- (10) PIERCE GB, NAKANE PK, MAZURKIEWICZ JE: The natural history of the malignant stem cell. In *Differentiation and Control of Malignancy of Tumor Cells* (Nakamura W, Ono T, Sugimura T, et al, eds.). The Fourth International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, Japan, November 1973. Univ Tokyo Press, 1974, pp 453-469
- (11) FRIEDWALD WF, ROUS P: Pathogenesis of deferred cancer: A study of the aftereffects of methycholanthrene upon rabbit skin. *J Exp Med* 91:459-484, 1950
- (12) REAGAN JW, WENTZ WB: Changes in the mouse cervix antedating induced cancer. *Cancer* 12:389-395, 1959
- (13) FOULDS L: Neoplastic development. In *Chemical Basis of Development* (McElroy WD, Glass B, eds.). Baltimore, The Johns Hopkins Univ Press, 1958, pp 680-700
- (14) BERENBLUM I, SHUBIK P: A new quantitative approach to the study of the stage of chemical carcinogenesis in the mouse's skin. *Br J Cancer* 3:383-391, 1949
- (15) RAICK AM: Cell differentiation and tumor-promoting action in skin carcinogenesis. *Cancer Res* 34:2915-2925, 1974
- (16) MOORE RA: The morphology of small prostatic carcinoma. *J Urol* 33:224-234, 1935
- (17) GROBSTEIN C, ZWILLING E: Modification of growth and differentiation of chorio-allantoic grafts of chick blastoderm pieces after cultivation at a glass-clot interface. *J Exp Zool* 122:259-284, 1953
- (18) FISHER B, FISHER E: Experimental evidence in support of the dormant tumor cell. *Science* 130:918-919, 1959
- (19) LEIGHTON J: *The Spread of Cancer*. New York, Academic Press, 1967
- (20) PIERCE GB: Differentiation of normal and malignant cells. *Fed Proc* 29:1248-1254, 1970
- (21) MOSCONA AA: Cell aggregation: Properties of specific cell ligands and their role in the formation of multicellular systems. *Dev Biol* 18:250-277, 1968
- (22) GARBER BB, MOSCONA AA: Reconstruction of brain tissue from cell suspensions. I. Aggregation patterns of cells disassociated from different regions of the developing brain. *Dev Biol* 27:217-234, 1972
- (23) MENDELSON MD: Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C3H mouse. III. The growth fraction. *J Natl Cancer Inst* 28:1015-1029, 1962
- (24) WYLIE CV, NAKANE PK, PIERCE GB: Degree of differentiation in nonproliferating cells of mammary carcinoma. *Differentiation* 1:11-20, 1973

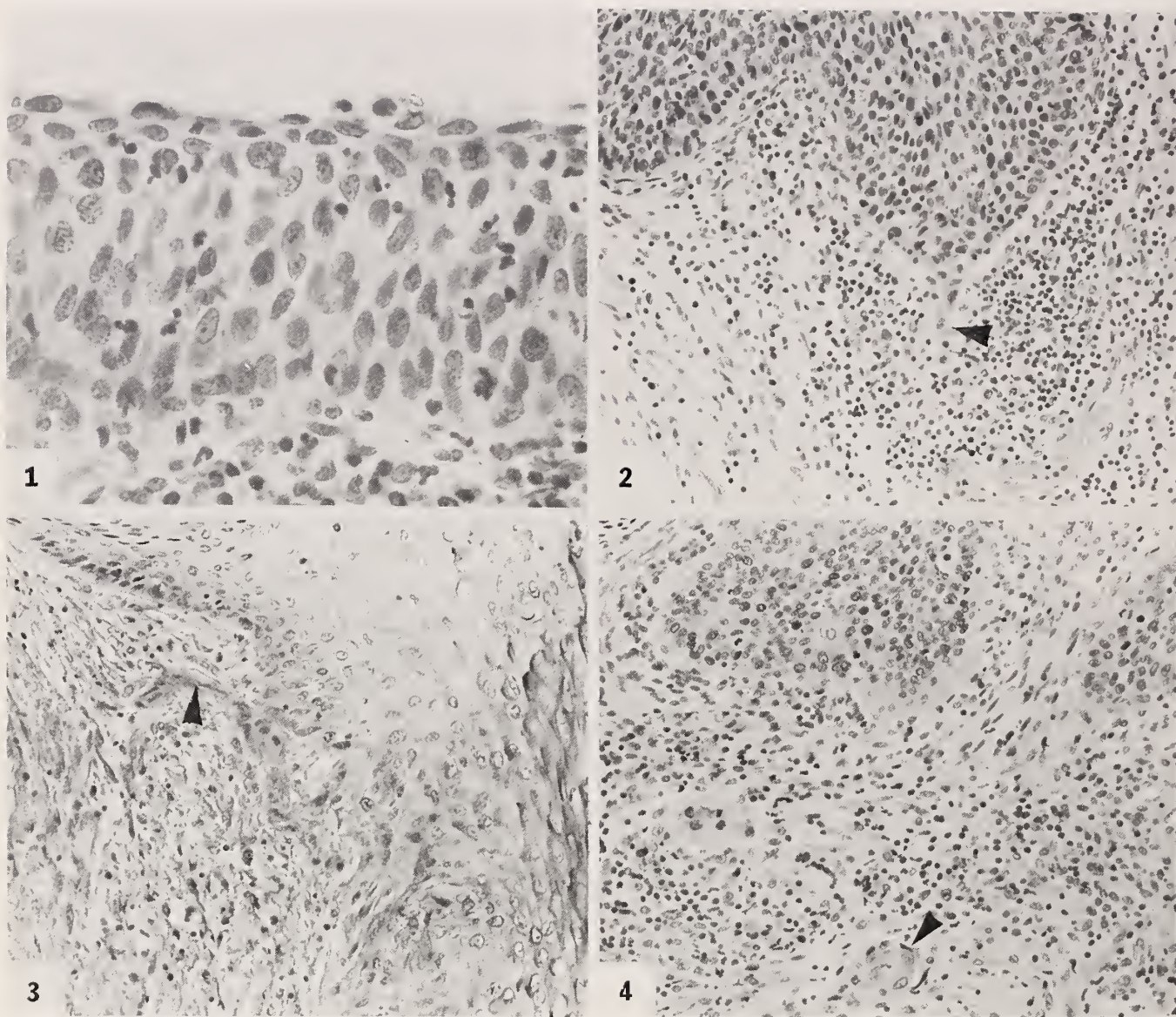


FIGURE 1.—Carcinoma in situ illustrating typical cytologic characteristics of malignancy but lacking invasion. $\times 528$

FIGURE 2.—Typical carcinoma in situ filling an endocervical gland. Bud of slightly more mature squamous epithelium has invaded through basement membrane into stroma (*arrow*). This is microinvasive carcinoma. $\times 211$

FIGURE 3.—Example of microinvasive carcinoma (*arrow*) originating from dysplastic squamous epithelium, better differentiated than carcinoma in situ. $\times 211$

FIGURE 4.—Microinvasive carcinoma to illustrate appearance of squamous elements in vascular channels (*arrow*). Note mature appearance in relationship to overlying carcinoma in situ. $\times 211$

III.
HOST DEFENSE MECHANISMS

Some Immunologically Based Reactions That Can Cause the Regression of Large Tumor Masses¹

Peter Alexander²

SUMMARY—A widely held view is that the immune reactions of the host directed against tumor-specific membrane antigens can only eliminate a relatively small number of tumor cells. There are, however, some therapeutic maneuvers, that under some conditions can cause the regression of large tumor masses to which immune factors make a critical contribution. Among these are the injection of complement, administration of double-stranded RNA and endotoxin, and the induction of inflammation.—*Natl Cancer Inst Monogr* 44: 105-108, 1976.

There are many reasons why isolated tumor cells or small aggregates of tumor cells do not give rise to a progressively growing tumor in vivo. Malignant cells are shed continually from a primary tumor into the bloodstream, yet frequently none of these cause metastases despite the fact that they are trapped in the lung. Immunologic host factors are important in the control of small masses of disseminated tumor cells. The destruction of subclinical masses of tumor is not, however, the phenomenon of spontaneous regression of cancer to which this symposium is directed. Spontaneous regression of clinically evident cancer involves the disappearance of billions of tumor cells.

It is frequently stated that the immune defenses of the host are capable of coping with thousands up to millions of tumor cells but cannot cause destruction of macroscopic tumor masses. This dogma is based on experimental studies in which animals that had been immunized intensively against a tumor and resisted a challenge with a million or fewer viable cancer cells developed a tumor when given larger inocula. To find out whether immune factors can cause the disappearance of well-established tumor masses we need animal models, because the phenomenon in man is too rare for systematic study. I believe an excellent model is about to be developed by Theilen et al. (1), who induced primary sarcomas in sheep with a feline sarcoma virus that had been adapted to transform sheep cells. After inoculation of virus-infected cells, autochthonous tumors appear within a few weeks and grow rapidly. The sheep react immunologically against antigens on these tumor cells, but in spite of a brisk humoral and cellular response, the tumor grows. However, spectacular spontaneous regressions of tumors weighing hundreds of grams frequently occur several weeks after the tumors have first become palpable and after an effective host immune reaction had been established. Why the tumors are not rejected by the host when they are relatively small and when there is a good immune response but are rejected when they are large seems to provide a most elegant model for the study of spontaneous regression of tumors in man. I must emphasize that when these tumors in the sheep regress, there is no sign of necrosis or of failure of blood supply.

We already know of a number of experimental procedures with an immunologic basis that can cause the disappearance of large amounts of primary or syngeneically grafted tumors in experimental animals. Though I do not believe that the mechanisms underlying these manipulations necessarily have a function in any of the spontaneous regressions observed in man, they provide examples of how immune mechanisms can contribute to the destruction of large amounts of tumor. If the conditions are right, it would appear that the dogma that immunity can take care of only minimal residual disease may not be true. Here I shall deal with treatments that have an immunologic basis; this does not say that I believe that immune mechanisms have the only, or even perhaps the most important, role in the spontaneous regressions that have been seen clinically.

INJECTION OF COMPLEMENT

Kassel and his colleagues (2) found that the injection of fresh serum caused temporary but striking regressions of large masses of spontaneously occurring lymphomas in cats and in AKR mice. The AKR mouse is genetically deficient in the C4 component of complement, and a detailed study showed that the antitumor action of the fresh serum was due to its providing C4. In the case of the cats, the necessary experiments were not done to prove that complement was involved in the observed regressions.

Patients presenting with acute leukemia frequently receive fresh blood to correct their anemia, and it is not uncommon to find that circulating leukemic blast cells disappear from the blood for several weeks after a transfusion. The experiments with the AKR mice suggest that the role of complement ought to be considered in such cases. The sera from patients with malignant disease are frequently anticomplementary; this could be genetic or due to the presence of circulating antigen-antibody complexes, and it is conceivable that a change in the availability of complement in the host may sometimes cause billions of circulating leukemia cells to disappear.

REGRESSIONS OF ESTABLISHED TUMORS INDUCED BY ENDOTOXIN AND DOUBLE-STRANDED RNA (dsRNA)

In 1866, a Viennese physician reported that he had observed dramatic regressions of some malignant tumors and that they appeared to coincide with infectious episodes (erysipelas). Some years later, Coley made a similar observation; he decided to treat patients with culture fluids from bacteria (these fluids contained, among many other components, endotoxins) and induced a number of striking antitumor effects in patients with gross inoperable cancer (3). It has not been demonstrated that endotoxin was the active principle in the clinical situation, but endotoxin alone was found to be effective against some animal tumors.

In 1936, Andervont (4) showed that large, primary, chemically induced skin tumors in mice regressed completely after systemic treatment with endotoxin. This is not a universal effect, and some experimental tumors (primary

¹ Presented at the Conference on Spontaneous Regression of Cancer, held at The Johns Hopkins Medical Institutions, Baltimore, Md., May 9 and 10, 1974.

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and transplanted) do not regress; Andervont noted that, in general, tumors growing in the skin were most responsive. The remarkable feature of the antitumor action of endotoxin is that this agent is most effective when directed against well-established tumors and is almost ineffective against small numbers of tumor cells. This, of course, distinguishes it from the normal cytotoxic chemical agents, which cure animals bearing a small tumor load but are generally ineffective against large and well-established primary or syngeneic tumors. When endotoxin is effective, the antitumor action is more dramatic than can be produced by any of the known anticancer chemotherapeutic substances.

The reason why endotoxin works in tumors only after they have become fully established and is inactive when given immediately after the injection of a few tumor cells is due to the fact that it does not act directly on the malignant cells in the tumor. The most readily observable effect is damage to the blood vasculature of the tumor. While damage of blood vessels in the tumor, contributed by the normal cells of the host, appears to be an essential component of the antitumor endotoxic actions, it is not by itself sufficient to cause permanent regressions. We (5) found that to obtain permanent regressions after treatment with endotoxin, it was necessary for the immune system of the host to be intact. In immunosuppressed mice, endotoxin still causes hemorrhage in the tumors, as a result of which the tumor shrinks in size temporarily but then rapidly regrows (text-fig. 1).

We (5) also observed a strict correlation between the responsiveness of syngeneic transplants growing intradermally or subcutaneously and the immunogenicity of the tumors as measured by standard transplantation criteria (table 1). The need for both hemorrhagic necrosis

TABLE 1.—Effect of dsRNA and endotoxin on various syngeneic tumors transplanted intradermally^a

Tumor	Antitumor effect of dsRNA	Immunogenicity of tumor
Lymphomas		
L5178Y (DBA/2)	+++	+++
L5178Y-M (DBA/2)	—	+
TLLX9 (C57BL)	+—	+—
TLC5 (CBA)	+—	+
SL2 (DBA/2)	+++	++
Gardiner (CBA)	+++	++++
Fibrosarcomas		
FS1 (C57BL)	++	+++
FS6 (C57BL)	++	+++
FS9 (CBA)	—	+

^a—no effect; +=slowing of growth; ++=temporary regression in 50% of the animals; +++=permanent regression in 50% of the animals.

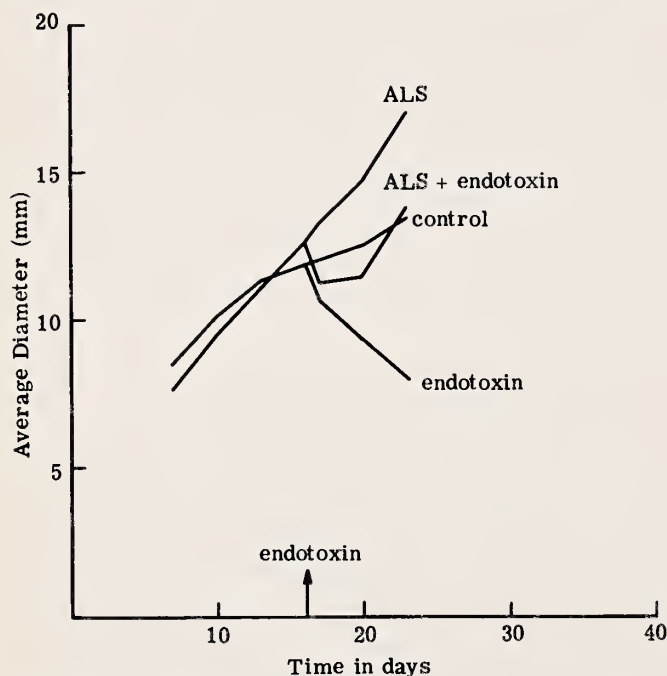
and an active immune response suggests that the action of endotoxin on tumors is due to a breakdown of the vascular structure, which allows the various immunologic effector mechanisms of the host to gain access to the tumor. Vascular damage alone can cause only a temporary slowing of tumor growth, but when this occurs in a host reacting to an antigenic tumor, the combined response results in the spectacular regressions that are observed.

An additional mechanism may also come into play due to the "activation" of macrophages. When macrophages are exposed to endotoxin, they undergo an unusual form of activation, as a result of which they become cytotoxic to malignant cells (6). This occurs both in vitro and in vivo; after injection of endotoxin into mice, the macrophages in the peritoneal cavity acquire the capacity to kill tumor cells in vitro (6). It is not yet clear how much this process contributes to the antitumor action of endotoxin in vivo, but in another situation (see below) we have clear evidence that activation of macrophages can induce the regression of relatively large amounts of tumor growing in the peritoneal cavity or in the lung.

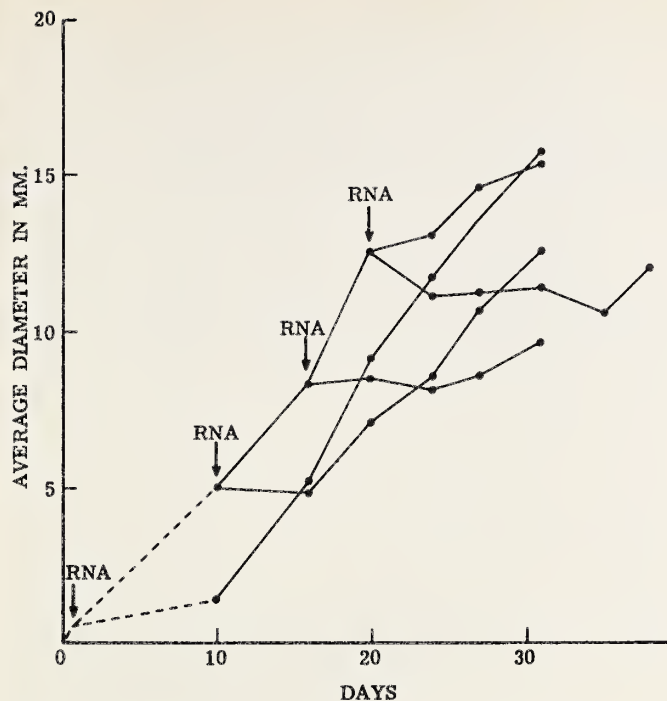
We found (5), to our surprise, that the characteristic antitumor action of endotoxin was closely mimicked by administration of dsRNA whether of viral origin or produced synthetically (i.e., polyI-polyC). Like endotoxin, dsRNA (text-fig. 2) has no direct action on tumor cells and causes regressions only when the tumors have become established. When given soon after the transplant of tumor cells, dsRNA was, in our experiments, ineffective against subcutaneous or intradermal lymphomas and sarcomas. The antitumor action of dsRNA requires the cooperation of the immune defenses of the host, and it also renders macrophages cytotoxic to tumor cells (6). In the in vivo antitumor action of endotoxin and dsRNA, we have a clear example of the disappearance of large tumor masses by a complex process in which the immunologic reaction of the host directed against the tumor plays an essential part.

IN VIVO ANTITUMOR ACTION OF "ACTIVATED" MACROPHAGES

I have already referred to the phenomenon in which macrophages from normal animals, after exposure to endotoxin or dsRNA, acquire the capacity to inhibit the growth of tumor cells in vitro. The same type of activation



TEXT-FIGURE 1.—Effect of immunosuppression by antilymphocyte serum (ALS) on the antitumor effect of endotoxin against a syngeneic lymphoma L5178Y inoculated intradermally into DBA/2 mice. Text-figure shows growth of the tumors in normal and ALS-pretreated mice and effect on the tumor of 10 μ g endotoxin injected ip 16 days after the transplant into control and ALS-treated mice.



TEXT-FIGURE 2.—Effects of injections of 100 μ g dsRNA ip (from a fungal virus) into C57BL mice at different times following an sc transplant of a syngeneic fibrosarcoma (FS1). Group A, dsRNA given 1 day after transplant; Group B, dsRNA given 10 days after transplant; Group C, dsRNA given 16 days after transplant; Group D, dsRNA given 20 days after transplant.

can also be achieved immunologically, if macrophages taken from an animal immunized against a specific antigen are exposed to the antigen. Macrophages then have the capacity to kill tumor cells, i.e., they become “activated.” This phenomenon occurs both in vitro and in vivo (7). By induction of activation of macrophages in vivo in this immunologic way, tumor cells in the peritoneal cavity (see table 2) and in the lung can be destroyed (8, 10). In the experiment illustrated in text-figure 3, mice were immunized with a strain of BCG (Glaxo), which is of comparatively low infectivity for mice; peritoneal macrophages, taken a week or more after immunization with this BCG, are not activated but become so on the addition of purified protein derivative (PPD, soluble protein from tubercle bacilli) (7).



TEXT-FIGURE 3.—Effect of 4 μ g soluble protein from tubercle bacilli (PPD), injected ip, on the growth of 4×10^6 syngeneic fibrosarcoma (FS6) cells inoculated ip into C57BL mice sensitized with 300 μ g of Glaxo BCG 14 days prior to inoculation of the tumor cell. Curves show percentage surviving (in groups of 10 mice) at different times. Treatment of mice indicated by the following lines: 1) Normal mice + tumor (—), 2) normal mice + tumor + PPD (.....), 3) BCG sensitized mice + tumor (---), 4) BCG sensitized mice + tumor + PPD (----).

Pretreatment of mice with BCG increased to a small extent their resistance to a subsequent challenge with the 2 tumors used by us. This is an example of the well-established phenomenon (9) that immunization with BCG increases the resistance of animals to a subsequent challenge with tumors. However, when PPD was administered after injection of tumor cells into the BCG-immunized mice, there was a dramatic effect: No tumors developed in any of the mice. In mice that had not been preimmunized with BCG, the addition of PPD to the sarcoma cells did not affect their growth in vivo. It must be stressed that in this experiment, an immunologic maneuver caused the rejection of 4×10^6 tumor cells. We conclude that activation of macrophages by exposure to an antigen (unrelated to the tumor) to which the animal had previously been rendered immune is another example of an immunologic process that can cause the elimination of large amounts of tumor at favorable sites such as the peritoneal cavity or the lung. This phenomenon does not apply equally to all tumors tested, and table 2 shows that the effect is much smaller with a lymphoma than with the sarcoma used in the experiment illustrated in text-figure 3.

TABLE 2.—Effect of PPD on growth of lymphoma cells (L5178Y) in DBA/2 mice immunized with BCG

Treatment protocol				Percent of mice surviving after injection of lymphoma cells		
Day 1	Day 8	Day 20	Day 21	25 days	26–49 days	50 days
None	None	10^4 L5178Y cells ip	None	90	10	0
None	None	10^4 L5178Y cells ip	PPD ip	100	0	0
BCG	BCG	10^4 L5178Y cells ip	None	60	40	0
BCG	BCG	10^4 L5178Y cells ip	PPD ip	30	20	50
BCG	BCG	10^4 L5178Y cells ip	PPD iv	60	40	0
BCG	BCG	10^4 L5178Y cells ip	PPD sc	40	60	0

REFERENCES

- (1) THEILEN J, HALL JG, PENDRY A, et al: Tumors induced in sheep by injecting cells transformed in vitro with feline sarcoma virus. *Transplantation* 17:152-155, 1974
- (2) KASSEL RL, OLD LJ, CARSWELL EA, et al: Serum-mediated leukemia cell destruction in AKR mice. *J Exp Med* 138:925-938, 1973
- (3) NAUTS HC, FOWLER GA, BOGATKO FH: A review of the influence of bacterial infection and of bacterial products (Coley's toxins) of malignant tumors in man. *Acta Med Scand (suppl 276)* 145:1-103, 1953
- (4) ANDERVONT HB: The reaction of mice and various mouse tumors to the injection of bacterial products. *Am J Cancer* 27:77, 1936
- (5) PARR IB, WHEELER LE, ALEXANDER P: Similarities of the anti-tumour actions of endotoxin, lipid A and double-stranded RNA. *Br J Cancer* 27:370-389, 1973
- (6) ALEXANDER P, EVANS R: Endotoxin and double stranded RNA render macrophages cytotoxic. *Nature [New Biol]* 232:76-78, 1971
- (7) EVANS R, ALEXANDER P: Mechanism of immunologically specific killing of tumour cells by macrophages. *Nature* 236:168-170, 1972
- (8) ALEXANDER P: Activated macrophages and the antitumor action of BCG. *Natl Cancer Inst Monogr* 39:127-133, 1973
- (9) OLD LJ, BENACERRAF B, CLARKE DA, et al: The role of the reticuloendothelial system in the host reaction to neoplasia. *Cancer Res* 21:1281-1300, 1961
- (10) PARR, IB, WHEELER LE, ALEXANDER P: Rejection of sarcoma cells at the site of an inflammatory reaction. Macrophages are not the only effector cells. *Cancer Lett* 1:49-53, 1975

Immune Surveillance—A Powerful Mechanism With a Limited Range^{1,2}

George Klein³

SUMMARY—There is excellent evidence for the belief that immune surveillance mechanisms prevent the outgrowth of potentially neoplastic cells induced by horizontally transmitted, ubiquitous, potentially oncogenic viruses, indigenous to natural populations. Polyoma virus in mice, Marek's disease in the chicken, *Herpesvirus saimiri* in the squirrel monkey, and the Epstein-Barr virus in man are examples. There is much less evidence for immune surveillance against chemically induced tumors. It is argued that surveillance against the virus-induced tumors may have evolved by the selective fixation of appropriate immune responsiveness (IR) genes. It is important to distinguish between the breakdown of an existing surveillance mechanism, e.g., by immunosuppression, and the lack of immune recognition, due to the deficiency of the IR gene equipment. Presently available in vitro lymphocytotoxicity tests are not yet developed to the point where they can reliably distinguish between these alternatives.—*Natl Cancer Inst Monogr* 44: 109-113, 1976.

I was particularly delighted to hear Dr. Pierce this morning (1). Although I am an immunologist and appear on a panel together with other immunologists, I think that it would be dangerous to assume that the host can only keep tumor cells under control by immunologic mechanisms. Dr. Pierce has given some excellent examples of other controls. They involved local, contactual, and long-range hormone controls as well. Using still another example, he showed us how certain tumor cells can differentiate to a point at which they become quiescent. This means that we cannot assume without direct evidence that dormant tumor cells are kept under control by immune mechanisms in any situation.

In spite of the impression that one may get from the literature, the number of demonstrably immunogenic tumors in vivo, as judged by rejection tests, is still rather small. Nearly all virus-induced experimental tumors are immunogenic and rejectable. A small, relatively selected group induced by chemical carcinogens, mainly aromatic hydrocarbons and azo dyes, are also detectably immunogenic and rejectable. There is an increasing number of chemically induced tumors that are not detectably immunogenic, however. There is often a tendency to sweep them under the carpet. Baldwin (2) probably tested the largest variety and found that the different kinds of tumors induced by acetylaminofluorene are not detectably rejectable in vivo. He also showed that several spontaneous rat carcinomas and sarcomas lacked detectable antigenicity in vivo. Nevertheless, lymphocytes of hosts that carried such tumors were cytotoxic to the same tumor cells in vitro. Taken together

with the discrepant cross-reactivity patterns obtained in vivo and in vitro, this raises doubts about the in vivo relevance of at least some in vitro reactions. It seems that some antigens demonstrated (or suggested) by the lymphocytotoxicity tests cannot serve as rejection targets in vivo. This also questions the extrapolation from in vitro tests to judgments on the rejection potential of human tumor patients. To express this in another way, more direct evidence will be required to show that human tumor antigenicity, suggested by the lymphocytotoxicity tests, corresponds to a rejection potential in vivo.

In systems in which tumor-associated antigens were conclusively shown to function as rejection targets, various escape routes are open to the neoplastic cells. Each tumor immunologist likes to make his favorite escape list. It can be quite long, and there is no rational reason to choose one of the possibilities as *the* most important mechanism. I shall show my favorite escape list in the next session. At this point, I would like to emphasize that one cannot speak of escape unless there is a clear barrier. We must start by asking what barriers exist under natural conditions.

As I have discussed in detail elsewhere (3), the best evidence for the existence of strong immunologic barriers against tumor growth (akin to a surveillance mechanism) comes from systems in which ubiquitous viruses infect most members of a given species and occasionally induce tumors in some of them. In these cases, genetically determined immune mechanisms have been fixed by evolution, securing the prompt recognition of the virally determined, tumor-associated antigens. I shall give you some examples.

Marek's disease, a herpesvirus-induced chicken lymphomatosis is one example [for review see (4)]. It used to be a great economic problem for the poultry industry until a vaccine was recently developed (HVT, an apathogenic, partially related turkey herpesvirus). Genetically determined resistance plays an important role. In some crosses, resistance is due to a single dominant gene, and there is good reason to believe that it acts via the immune system. Genetically resistant chicks develop a self-limiting, benign disease instead of the progressive malignant lymphoma.

Polyoma virus in wild mice is another case. It is often said that polyoma is more or less a laboratory artifact, a plaything for molecular biologists who like to work with small viruses that can change normal into potential tumor cells in vitro with great efficiency. The epidemiologic study by Huebner (5) has shown that polyoma virus-infected wild mice do not develop any tumors in the laboratory. It was therefore assumed that the virus cannot cause any tumors under natural conditions. Contrary to these views, I tend to regard polyoma as a prototype example of a naturally occurring, potentially oncogenic virus that lives in a generally harmless, symbiotic association with its host species, the mouse, because the mouse has been selected for the prompt recognition of a polyoma virus-induced cell surface change as an antigen. We usually refer to this antigen as TSTA (tumor specific transplantation antigen). That term has emerged from the experimental finding that

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immunization of adult mice against polyoma virus or against polyoma-transformed tumor cells protects against the isografting of small or moderate cell numbers from established, polyoma virus-induced tumors, but not against cells from other tumors [for review, *see* (6)]. In vivo tumor induction is possible only if newborn mice of virus-free strains are exposed to the agent during the first 24 hours after birth. Apparently, the growth of in vivo-transformed, potentially neoplastic cells can proceed in neonatally inoculated mice to a point of no return, i.e., reach a size or achieve a state of vascularization that puts it beyond the reach of the rejection reaction notwithstanding the fact that the host of the primary tumor is perfectly capable, by this time, of resisting the subcutaneous reinoculation of small cell numbers from autochthonous or syngeneic polyoma tumors (6).

With this background, it becomes understandable why wild mice do not develop polyoma tumors. Since most adults are infected, mothers transmit antibodies passively to the young and protect them from the oncogenic action of the virus during the first critical 24 hours. Thereafter, the immune system matures quickly to a point at which the young mice can respond actively to TSTA and thereby reject their own incipient tumor cells. Naturally, this requires an intact immune system. Neonatally thymectomized, antilymphocyte serum-treated, or X-irradiated mice develop a large number of tumors, even if inoculated as adults (7, 8). The response of nude mice is even more dramatic (9).

A third case in point is the Gross mouse leukemia virus. This agent was originally isolated from the AKR mouse strain, developed by continuous inbreeding and selection for high leukemia incidence. The AKR mice cannot be protected against the transplantation of AKR leukemia cells by the preimmunization procedure that is so effective in the polyoma system (10). This is not due to a lack of a tumor-associated antigen, however. If leukemia is induced experimentally by Gross virus in a strain that does not transmit it vertically, e.g., C3H, previously uninfected syngeneic recipients can be readily immunized against the isografting of established lymphoma cells induced by the Gross virus (11). Concomitantly, cytotoxic antibodies are formed against a Gross virus-determined cell-surface antigen (GCSA). They can be used to demonstrate that GCSA is also present on the surface of the apparently non-immunogenic AKR leukemia cells.

The incisive analysis of Lilly and Pincus (12) elucidated some of the multiple genetic factors that favor leukemia development in AKR mice. At least three different genes were fixed by inbreeding and selection. One is designated *Akv-1*, is localized in the neighborhood of the albino (*C*) locus, and appears to represent the integration site of the Gross virus, or more appropriately, one of the two known integration sites in this strain.

Corresponding integration sites exist, although with a different localization, in other high leukemia strains. The C3H/Fg strain carries three viral genes, C58 mice carry four genes, and so on; the number of viral genomes and their integration sites thus differ between different high leukemia lines, but each strain transmits "its" viral genes faithfully.

A completely different genetic system, illustrated by the *Fv-1* locus, influences the spontaneous production of certain C-type viruses. It exists in two allelic forms, *n* and *b*. So-called N-tropic viruses (e.g., the Gross virus) are produced with a much greater efficiency in *nn* than in *nb* or *bb* hosts. The AKR mice are *Fv-1ⁿFv-1ⁿ*. Inbreeding has

thus fixed the appropriate amplification gene for the Gross virus. A third locus, perhaps of the greatest interest for immunologists, is *Rgv-1* (12). This influences the likelihood of leukemic development. Most mouse strains contain the *r* allele that conveys a relative resistance against Gross virus-induced leukemia, even in mice that carry the integrated viral genome and contain the appropriate spreading factor. The AKR mice carry the *s* allele that determines susceptibility. Although there is no conclusive evidence yet that *Rgv-1* is an immune responsiveness (IR) gene, this is no doubt the most likely explanation, since it is localized within the *K* region of the *H-2* complex, the site of several powerful IR genes. Also, resistance is dominant in F₁ hybrids, as expected for an IR gene. C3H, the strain to which Gross transmitted the virus experimentally, carries the same *H-2* complex (*H-2^k*) and the same *H-2*-linked *Rgv-1^s* allele as AKR. It is therefore possible that the inbreeding and selection of AKR for high leukemia incidence has led to the fixation of a specific genetic deficiency, comparable with an unresponsiveness gene, which thereby abolishes the normal resistance against the outgrowth of the virus-transformed leukemia cells.

Another *H-2*-linked gene that can influence the probability of an oncogenic process was found by Mühlbock and Dux (13) in the course of their studies on mammary tumors induced by the milk agent, murine mammary tumor virus (MuMTV). By comparing the tumor incidence in MuMTV-infected C57BL mice and in a number of *H-2* and non-*H-2*-substituted congenic resistant sublines, they demonstrated the existence of an *H-2^b*-linked resistance gene. This gene is derived from C57BL, a strain that has been selected for a low incidence of mammary carcinoma. In this case, it is even less certain that the resistance factor acts via an immune mechanism; among other things, it is localized near the *D* end of the *H-2* locus. This is one of several possibilities, however.

Known IR genes are partly linked to the major histocompatibility complex (MHC) of the species and partly scattered over the whole genome (14). Particularly, the MHC-linked loci appear to act at the T-cell level, whereas others may influence T-B cell cooperation or the B cell-dependent arm of antibody formation. In some cases, there is a rather clear-cut, all-or-none, difference between responsiveness and unresponsiveness, whereas in other cases the difference is more a question of timing or of certain qualitative or quantitative aspects of the response.

In the lymphotropic simian *Herpesvirus saimiri* system, virtually all adult squirrel monkeys, the natural host species, harbor the virus and make antibodies. They are not known to develop any disease due to this virus. Inoculation of the virus into some other New World monkeys, e.g., marmosets or owl monkeys, leads to lethal lymphoproliferative neoplasia in 100% of them (15). The disease consists of a polyclonal growth, mainly of T cells. Studies on the antibody formation against early and late viral antigens (16) showed that both the naturally resistant and the susceptible experimental species are capable of responding, but the response of the latter is delayed to a point at which malignant growth is already a fact. It is likely that the natural host was selected for resistance against neoplasia induced by this virus. In all probability, it has evolved multiple controls. Some of these must be immunologic, enabling the host to respond promptly and efficiently to the viral and virally determined cellular antigens. This would not be the case in the susceptible species that does not encounter this virus normally.

The Epstein-Barr virus (EBV) probably represents an analogous situation in man (17). Infectious mononucleosis (IM) was mentioned earlier during this meeting as an example of a potentially malignant but normally self-limiting disease. It has been conclusively established that IM is caused by a primary EBV infection (18, 19). Cell lines can be established from IM patients and from other seropositive donors that carry the viral genome.

These lines carry the viral genome, usually in the form of multiple copies per cell. They are "immortalized" (20), i.e., are capable of continued proliferation in vitro, unlike normal B cells that have a limited life-span. When tested in nude mice or in other immunosuppressed animals, they grow progressively as malignant lymphomas and kill their hosts (21-23). Thus they have, at least, a malignant potential. Similar potentially malignant lines can be created at will by addition of EBV to cord blood lymphocytes (24-26). Since EBV is not vertically transmitted, cord blood cells represent the easiest source of virus-free transformed cells.

Recently, it was found that EBV can induce lymphomas directly in marmosets and in owl monkeys (27, 28). These lymphomas carry the viral genome and the virally determined nuclear antigen (EBNA).

There is thus hardly any question about the oncogenic potential of EBV. Nevertheless, 80-90% of us harbor this virus over a lifetime without any obvious ill effect. Our relationship to this virus is probably comparable with the symbiotic association between polyoma virus and wild mice, or *H. saimiri* and squirrel monkeys. If this is correct, we have been selected during our evolutionary history to deal with the potentially malignant cells induced by this virus. The intense transformation of T lymphocytes into blast cells, upon confrontation with autologous EBV-carrying cell lines, followed by the differentiation of killer cells, may reflect the postulated reaction (29). In this connection, it is interesting to note that the atypical lymphocytes which appear during acute IM are T cells (30). Recent preliminary evidence indicates (Svedmyr EA, Jondal M: Personal communication) that they exert a specific killing effect against EBV genome-carrying, in contrast to EBV genome-free, cell lines.

Elsewhere, I have developed the concept (31) that recognition of virally changed lymphoid cells with a malignant potential may be one of the most important functions of the immune surveillance system. The autologous lymphocyte stimulation induced by EBV-transformed lines, mentioned above, may be one expression of this. Conceivably, a rearrangement of the serologically defined and the lymphocyte-recognized components of the MHC may be involved in the malignant transformation of lymphoid cells. This hypothesis is based on the fact that these receptors are involved in the triggering of DNA synthesis in the normal lymphocyte upon the receipt of appropriate stimuli. Virus-induced rearrangements may therefore lead to a dysfunction, with disobedience to the normal growth-controlling signals and malignancy as the result. If so, a sensitive surveillance system protecting against cells characterized by such rearrangements would make good sense.

I shall now proceed to the question of the relationship between EBV and human cancer. Although the virus is widespread and the majority of normal healthy adults in any given human population are seropositive, i.e., have been and probably are virus infected, the viral genome is associated with only two forms: African Burkitt's lymphoma and nasopharyngeal carcinoma of diverse

geographic origin. The serologic picture (32, 33) is unique in patients of these two categories, with 100% EBV seropositivity and a tenfold elevation of the geometric mean titer. Tests designed to trace the viral genome and its antigenic footprints, EBNA, have given an even clearer and more discriminating picture (34-38). The nuclear antigen was regularly present in all EBV-DNA-positive biopsy specimens and cell lines and was equally regularly absent from genome-negative lines and biopsy specimens. Among 83 biopsy specimens of African Burkitt's lymphoma, 81 or 97% were positive for EBV genome by these criteria. Multiple genome equivalents were present per cell, 38 on an average. The mean and the range were closely similar to the number of genomes in EBV-positive, nonproducer, lymphoblastoid cell lines in vitro. None of 7 non-African Burkitt's lymphomas tested contained detectable EBV genome or EBNA. The same was true for the cases of Hodgkin's disease, chronic lymphatic leukemia, lymphocyte lymphoma, and various leukemias so far tested.

It is conceivable that most of the African Burkitt's lymphomas have an EBV-associated etiology, whereas non-African Burkitt's or Burkitt-like lymphomas arise by different mechanisms. Morphologic discrimination between Burkitt's and non-Burkitt's lymphomas is difficult and often subjective. Etiologic differences could be well imagined in spite of certain histologic or cytologic similarities. If that would be the case, the 3% EBV genome-negative cases found in Africa might correspond to the genome-negative cases all over the world. The genome-positive lymphomas would be superimposed on this background in the high endemic areas and might represent an essentially different disease. It is important to remember that African Burkitt's lymphomas show the most significant time-space clustering described for any human neoplasms (39), whereas there is no convincing evidence for time-space clustering of Burkitt-like lymphomas outside Africa (40).

The ubiquitousness of EBV and the widespread seropositivity of human populations has led to the frequently expressed view that the virus is merely a passenger in the lymphoma cells. Its predilection for human lymphoid cells would lead to an increased antigenic load and to increased antibody titers as the lymphoma grows. With the advent of the genome-localizing methods, this hypothesis has become untenable, however. The passenger hypothesis cannot explain why no lymphoproliferative malignancies have been found so far, *except* African Burkitt's lymphoma, that would carry the viral genome. This argument can be sharpened further by the following consideration:

We have established EBV genome and EBNA-negative cell lines from an exceptional, EBV genome-negative African lymphoma (representing the 3% negatives mentioned above) and from a genome-negative American Burkitt's lymphoma. Both lines have B-cell characteristics. They are designated BJA-B (41) and Ramos (Klein G, Giovanella G: Unpublished data), respectively. Both lines have been derived from EBV-seropositive donors. Nevertheless, there is no evidence that they have picked up any EBV in vivo. It could still be argued that the viral genome is present at subthreshold levels. Such a situation would differ in two respects from all known EBV-carrying lines, however, since they all contain the nuclear antigen *and* multiple copies of the viral genome as well. Rather than postulating such a difference, it is more reasonable to assume that the viral genome is not present in these lines, certainly not in its usual form.

It could then be argued that perhaps these cells were resistant against EBV infection. Both cell lines could be successfully infected with EBV in vitro, however, and one was permanently converted into an EBV-positive subline [(42); Clements G: Unpublished data]. All this makes the passenger hypothesis most unlikely. The two main alternatives that remain are the following:

The conclusion seems inescapable that the viral genome must be present in the genome-positive African Burkitt's lymphomas from the very inception of the tumor. Our collaborative work with Fialkow (43) has shown that Burkitt's lymphoma is always the proliferation of a single cell clone, even if multifocal. According to the first alternative, the African patient would develop the same dormant, EBV-carrying, potentially immortal cells as EBV-carrying normal individuals. Whereas the normal individuals can keep all potential transformants under control, presumably by immunologic mechanisms, one clone would slip through the control barrier in the African Burkitt's lymphoma patient, either because the control is inefficient for genetic or environmental reasons and/or because the clone is urged on by some other proliferation promoters. It must be emphasized that in spite of its relatively high endemic occurrence, African Burkitt's lymphoma is still a rare disease and, even in the Burkitt's patient it is only one clone that manages to escape. The relatively good curability of the tumor by chemotherapy in a substantial fraction of the patients (44), and the rare but well-documented spontaneous regressions (45) demonstrate, furthermore, that even the lymphoma patient may have considerable immunologic reactivity left, although the immunologic response has apparently not acted with sufficient intensity or in the right way at the right time.

According to the second alternative, this is not the whole explanation, since the dormant, EBV-carrying cells are not yet fully malignant. Some additional change would be required, due to, for example, another virus or a second cellular change, before the cells become fully capable of autonomous growth.

At the present time, we cannot distinguish between these alternatives. Perhaps a close comparison between the properties of EBV-carrying cells derived from normal individuals and from Burkitt's lymphomas may give some clues.

As already mentioned, there is ample evidence for a relatively strong immune response against Burkitt's lymphoma cells in the African patient. In addition to the phenomena Dr. Ziegler reviewed (46), there are also a number of tumor-associated antibody reactions. Whether there are similar tumor-associated immune reactions in the many other human tumors that we are currently discussing is open to question. It is important to distinguish between tumors in which there is evidence of rejection-inducing antigens and in which the malfunctioning of the effector mechanism may have contributed to the original development of the tumor, and systems in which the tumor cells are not recognized immunologically in the first place. Immunotherapeutic approaches will face a very different situation in the two cases. In the latter case, the problem is not how to strengthen an established response or how to counteract blocking or enhancing factors, but how to render genetically unresponsive animals responsive to a given antigen.

REFERENCES

- (1) PIERCE GB, FENNELL RH JR: Latent carcinoma and carcinoma in situ. *Natl Cancer Inst Monogr* 44:99-102, 1976
- (2) BALDWIN RW: Immunological aspects of chemical carcinogenesis. *Adv Cancer Res* 18:1-75, 1973
- (3) KLEIN G: Immunological surveillance against neoplasia. *Harvey Lect* 69:71-102, 1975
- (4) NAZERIAN K: Marek's disease: A neoplastic disease of chickens caused by a herpesvirus. *Adv Cancer Res* 17:279-315, 1973
- (5) HUEBNER RJ: Tumor virus study systems. *Ann NY Acad Sci* 108:1129-1148, 1963
- (6) SJÖGREN HO: Transplantation methods as a tool for detection of tumor specific antigens. *Prog Exp Tumor Res* 6:289-322, 1965
- (7) GAUGAS JM, ALLISON AC, CHESTERMAN FC, et al: Immunological control of polyoma virus oncogenesis in mice. *Br J Cancer* 27:10-17, 1973
- (8) LAW LW, DAWE CJ: Influence of total body X-irradiation on tumor induction by parotid tumor agent in adult mice. *Proc Soc Exp Biol Med* 105:414-419, 1960
- (9) ALLISON AC: Immunological surveillance against tumour cells. In *Scientific Foundations of Oncology* (Symington T, Carter RL, eds.). London, Heinemann Medical Books, 1975, pp 521-571
- (10) PASTERNAK G: Antigens induced by the mouse leukemia viruses. *Adv Cancer Res* 12:1-99, 1969
- (11) KLEIN G, SJÖGREN HO, KLEIN E: Demonstration of host resistance against isotransplantation of lymphomas induced by the Gross agent. *Cancer Res* 22:955-961, 1962
- (12) LILLY F, PINGUS T: Genetic control of murine viral leukemogenesis. *Adv Cancer Res* 17:231-277, 1973
- (13) MÜHLBOCK O, DUX A: Histocompatibility genes (the H-2 complex) and susceptibility to mammary tumor virus in mice. *J Natl Cancer Inst* 53:993-996, 1974
- (14) BENACERRAF B, KATZ DH: The histocompatibility-linked immune response genes. *Adv Cancer Res*. In press
- (15) DEINHARDT FW, FALK LA, WOLFE LG: Simian herpesviruses and neoplasia. *Adv Cancer Res* 19:167-205, 1974
- (16) KLEIN G, PEARSON G, RABSON A, et al: Antibody reactions to herpesvirus saimiri (HVS)-induced early and late antigens (EA and LA) in HVS-infected squirrel, marmoset and owl monkeys. *Int J Cancer* 12:270-289, 1973
- (17) KLEIN G: Immunological surveillance against tumors. In *Annual Symposium on Tumor Immunology* (Cumley RW, Abbott M, McCay J, eds.). Austin, Univ Texas Press, 1975, pp 21-54
- (18) EVANS AS, NIEDERMAN JC: Epidemiology of infectious mononucleosis. A review. In *Oncogenesis and Herpesviruses* (Biggs RM, de-Thé G, Payne LN, eds.). Lyon, International Agency for Research on Cancer, 1972, pp 351-356
- (19) HENLE G, HENLE W, DIEHL V: Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. *Proc Natl Acad Sci USA* 59:94-101, 1968
- (20) MILLER G: Human lymphoblastoid cell lines and Epstein-Barr virus: A review of their interrelationships and their relevance to the etiology of leukoproliferative states in man. *Yale J Biol Med* 43:358-384, 1971
- (21) ADAMS RA, FOLEY GE, UZMAN BG, et al: Leukemia: Serial transplantation of human leukemic lymphoblasts in the newborn Syrian hamster. *Cancer Res* 27:772-783, 1967
- (22) LEVIN AG, FRIBERG S JR, KLEIN E: Xenotransplantation of a Burkitt lymphoma culture line with surface immunoglobulin specificity. *Nature* 222:997-998, 1969
- (23) SOUTHAM CM, BURCHENAL JH, CLARKSON B, et al: Heterotransplantability of human cell lines derived from leukemia and lymphomas into immunologically tolerant rats. *Cancer* 24:211-222, 1969
- (24) HENLE W, DIEHL V, KOHN G, et al: Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. *Science* 157:1064-1065, 1967
- (25) MILLER K, ENDES JF, LISCO H, et al: Establishment of lines from normal human blood leukocytes by co-cultivation with a leukocyte line derived from a leukemic child. *Proc Soc Exp Biol Med* 132:247-252, 1969
- (26) POPE JH, HORNE MK, SCOTT W: Transformation of foetal human leukocytes in vitro by filtrates of a human leukaemic cell line containing herpes-like virus. *Int J Cancer* 3:857-866, 1968
- (27) EPSTEIN MA, HUNT RD, RABIN H: Pilot experiments with EB virus in owl monkeys (*Aotus trivirgatus*). I. Reticuloproliferative disease in an inoculated animal. *Int J Cancer* 12:309-318, 1973
- (28) SHOPE T, DECHAIRO D, MILLER G: Malignant lymphoma in cotton top marmosets after inoculation with Epstein-Barr virus. *Proc Natl Acad Sci USA* 70:2487-2491, 1973

- (29) SVEDMYR EA, DEINHARDT F, KLEIN G: Sensitivity of different target cells to the killing action of peripheral lymphocytes stimulated by autologous lymphoblastoid cell lines. *Int J Cancer* 13:891-903, 1974
- (30) SHELDON PJ, HEMSTED EH, PAPAMICHAIL M, et al: Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet* 1:1153-1155, 1973
- (31) KLEIN G: Epstein-Barr virus (EBV)-induced transformation of human lymphoid cells and immunosurveillance against lymphoma development. *Ann Immunol (Paris)*, 124C:391-405, 1973
- (32) HENLE G, HENLE W, CLIFFORD P, et al: Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. *J Natl Cancer Inst* 43:1147-1158, 1969
- (33) HENLE W, HENLE G, Ho H-C, et al: Antibodies to Epstein-Barr virus in nasopharyngeal carcinoma, other head and neck neoplasms, and control groups. *J Natl Cancer Inst* 44:225-231, 1970
- (34) ZUR HAUSEN H: Epstein-Barr virus in human tumor cells. *Int Rev Exp Pathol* 11:233-258, 1972
- (35) KLEIN G: Studies on the Epstein-Barr virus (EBV)-genome and the EBV-determined nuclear antigen in human malignant disease. Cold Spring Harbor Symposium on Quantitative Biology XXXIX:783-790, 1975
- (36) LINDAHL T, KLEIN G, REEDMAN BM, et al: Relationship between Epstein-Barr virus (EBV) DNA and the EBV-determined nuclear antigen (EBNA) in Burkitt lymphoma biopsies and other lymphoproliferative malignancies. *Int J Cancer* 13:764-772, 1974
- (37) PAGANO JS, CHIEN HH, LEVINE P: Absence of Epstein-Barr viral DNA in American Burkitt's lymphoma. *N Engl J Med* 289:1395-1399, 1973
- (38) REEDMAN BM, KLEIN G, POPE JH, et al: Epstein-Barr virus-associated complement-fixing and nuclear antigens in Burkitt lymphoma biopsies. *Int J Cancer* 13:755-763, 1974
- (39) MACMAHON B: Epidemiologic aspects of acute leukemia and Burkitt's tumor. *Cancer* 21:558-562, 1968
- (40) LEVINE PH, SANDLER SG, KOMP DM, et al: Simultaneous occurrence of "American Burkitt's lymphoma" in neighbors. *N Engl J Med* 288:562-563, 1973
- (41) KLEIN G, LINDAHL T, JONDAL M, et al: Continuous lymphoid cell lines with B-cell characteristics that lack the Epstein-Barr virus genome, derived from three human lymphomas. *Proc Natl Acad Sci USA* 71:3283-3286, 1974
- (42) KLEIN G, SUGDEN B, LEIBOLD W, et al: Infection of EBV-genome negative and positive human lymphoblastoid cell lines with biologically different preparations of EBV. *Inter-virology* 3:232-244, 1974
- (43) FIALKOW PJ, KLEIN G, GARTLER SM, et al: Clonal origin for individual Burkitt tumours. *Lancet* 1:384-386, 1970
- (44) CLIFFORD P, SINGH S, STJERNSWÄRD J, et al: Long-term survival of patients with Burkitt's lymphoma. An assessment of treatment and other factors which may relate to survival. *Cancer Res* 27:2578-2615, 1967
- (45) BURKITT DP, KYALWAZI SK: Spontaneous remission of African lymphoma. *Br J Cancer* 21:14-16, 1967
- (46) ZIEGLER JL: Spontaneous remission in Burkitt's lymphoma. *Natl Cancer Inst Monogr* 44:61-65, 1976

Neoplastic Disease and Tumor Immunology From the Perspective of Host-Parasite Relationships¹

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SUMMARY—Neoplasia is considered within the biologic framework of host-parasite relationships, and the arguments favoring this perspective are discussed. By viewing neoplastic processes as dynamic interactions between transformed clones, host defense mechanisms, and the environmental circumstances impinging directly on the neoplastic event, the experimentalist and clinician are better prepared conceptually for a balanced assessment of all factors determining the development and outcome of confrontations with neoplastic clones. The frame of reference placing progressive neoplasia into the category of host-parasite associations also prepares the ground for technical approaches to the study and observation of oncologic phenomena appropriate to their essential nature. The correct evaluation and most promising future development of the current emphasis on immunologic parameters of neoplasia demands especially an awareness of the role of immunologic variables in other host-parasite relationships.—*Natl Cancer Inst Monogr* 44: 115-122, 1976.

From what we have heard at this conference, two points have emerged quite clearly: The phenomenon of spontaneous regression of cancer is real, and it is very rare. One theme for my comments now and this afternoon will be that by studying the nature of the exception, spontaneous regression, we may learn to understand the rule, the multifaceted and dynamic entity of neoplastic disease.

In this presentation, I want to link the preceding session on "Host Defense Mechanisms" with the upcoming workshop on "Speculation, Hypotheses, and Theoretical Considerations" of the defenses evoked against neoplasia and the possible mechanisms of spontaneous cancer disappearance. There is good reason for attempting this bridge, and indeed, the usual frontal format of scientific communication—the presentation of data followed by a cautious analysis of their implications—is hardly possible in this area. Our knowledge of how the organism meets the challenge of neoplastic cells is still far too fragmentary, and based far too much on test systems that are coming to be recognized as artifactual, to offer explanation for the riddle of unexpected remission. Even the major parameters governing the dynamic confrontation between host and transformed cell remain to be elucidated. This paucity of pertinent information would compromise any effort at present to ascribe responsibility for atypical turns in the course of malignant disease to unique host reactions.

What we *can* do with the scattered observations before us is to formulate more focused questions and to construct operative assumptions and experimental approaches with which to attack the problem in the future. For this, we require a conceptual structure. My purpose today is to show the validity, indeed the necessity, of our viewing the

interaction between neoplastic cells and their host, and in particular, the occasional spontaneous regression of tumors in the context of host-parasite associations. In creating this frame of reference, we must, of course, base our deliberation on the available experimental and clinical evidence. We must also, however, play with ideas, entertain possibilities of major changes in the directions and interpretation of current research in tumor immunology, and essay the most hazardous of all forms of scientific dialogue, controlled speculation.

THOUGHT AND SEMANTICS

We must note at the outset that it is only in recent years that tumor-bearing animals have come to be viewed truly as *hosts* confronted with a population of pathogenetic cells sufficiently alien and independent to warrant the designation *parasite*, and capable of interacting dynamically with these challenging pathogens; i.e., it is only recently that we have come to view neoplasia as a host-parasite relationship (1-3). What is the substantive significance of the development of this perspective and, for that matter, of any emphasis on conceptual frameworks in sciences dealing with cognate phenomena?

I am well aware of the fact that many experimental biologists tend to have little patience with semantics and philosophy and with all that is subsumed by the terms. The impatience is understandable: Prepared guidelines to the explanation of experimental and clinical findings can easily lead to pigeonholing and to an artificial freezing of dynamic situations. Yet the formulation of research directions involves more than intuition. The design of experiments is, perforce, strongly influenced by the tacit assumptions of the investigator, and these, in turn, are qualified and faceted by the lexicon he uses; it is not possible to divorce ideation from language. Erroneous terminology and conceptualization invariably blur the focus of investigation. A correct use of terms and a well-formulated hypothetical infrastructure can, in contrast, measurably advance progress. Thus, whereas preconception, facile orthodoxy, and the forcing of new findings into a prepared ideologic matrix are anathema to the advancement of knowledge, we ignore at our peril the role of motivating hypothesis and of the use of descriptive language in the workings of natural science.

In developing the framework of study of host-parasite relationships for the analysis of defense mechanisms against progressive neoplasia, we must, accordingly, remember that the nuances of the ideas we shall entertain and the vocabulary we shall use derive from other areas of biology and that their application to our problem carries with it an existing import. Awareness of their origin and of their substantive implications can aid us appreciably in directing our future research efforts toward an understanding of key events in host-tumor associations and in viewing afresh information already developed in the light of a cohesive perspective.

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DEVELOPMENT OF THE VIEW OF PROGRESSIVE NEOPLASIA AS A HOST-PARASITE ASSOCIATION

In designating neoplasia as a host-parasite relationship, we visualize the apposition between a population of transformed autochthonous (and, in experimental models, syngeneic) cell variants with the cells of the host that specifically constitute its resistance apparatus and, secondarily, with all the host tissues that are recruited into a defensive position in the course of an ongoing interaction. To exploit to full advantage this perspective of malignant disease as a host-parasite relationship, we should consider the ontogeny of the concept, the nature of the observations that demanded its adoption, the reasons to expand and modulate it as new information developed, and its present validity and limitations.

Until quite recently, students of cancer saw the neoplastic cell as a pathologic condition and perhaps as a pathogenetic factor, but not truly as an interactant. The fate of neoplastic cells and of the organism in which they proliferate and the varied manifestations of the neoplastic process—malignancy, invasiveness, and metastasis—were held to be largely or exclusively the expressions of intrinsic properties of the transformed clones. It has, of course, long been realized that neoplastic cells are dependent to different extents for their proliferative ability on the normal physiologic status of the tumor bearer. However, it was not until the last few years that account was taken of the organism as an active participant, capable of reacting specifically to the neoplastic threat and of contributing significantly to the determination of the course of the disease. Neither was the possibility that extrinsic and intrinsic environmental parameters can impinge on a neoplastic process considered seriously as a major determinant of potentials of aggressive action by the neoplastic parasite and of defensive reactions by the host.

This restricted perception of the natural history of neoplasia is not surprising. It had its parallel in the area of infectious diseases, and it is convenient and safe. The bitter struggle at the end of the last century by the early proponents of the "germ theory" of disease for acceptance of a microbial etiology of infection resulted in a rigidly monistic view of disease causation which came to influence all of medicine. Suggestion of a multifaceted determination of the consequence of contagion, by which host and environmental factors interact with factors setting the pathogenic potentials of the parasite, appeared as a dangerous digression from the definitive causal role of microorganisms and as a pathway of return to the heresy of spontaneous generation of disease. When the incrimination of bacteria and of other Protista in a variety of pathologies became indisputable, microorganisms were soon assigned the *sole* function in the pathogenesis of infections. It followed only naturally that the same narrow, specific causation was postulated as well for immunogenesis: A given microorganism causes a given disease by virtue of its intrinsic pathogenic properties, and resistance and recovery are brought about by immunologic reactions initiated solely by that microorganism.

The central position of infectious diseases in medicine during the early decades of this century made it unavoidable that a similarly singular viewpoint should prevail generally. In the area of malignancy, the neoplastic cell thus came to be considered as *the* only important element in the natural history of cancer; the tumor bearer was an essentially passive entity; and environmental circumstances might play a part, at the most, in the epidemiology of malignant diseases. The fact that in some extreme cases of both

microbial and neoplastic disease, the parasitic cell by itself sets the picture appeared to validate this simplistic conception. It was given further support by the difficulties encountered by investigators who suspected a genuine participation of host and environment in various disease states but sought in vain to pinpoint these effects and, on the other hand, by the successes of prophylactic and therapeutic interference attempts aimed solely at the parasite.

It was only with the growing recognition during the past two to three decades that the properties of pathogenic cells cannot by themselves explain the variegated events of infectious and neoplastic diseases, that attention began to shift to host and to environment as cofactors with the parasite in the complex and shifting equilibria that characterize the fluctuating course of illness, recovery, and immunity.

In the field of neoplastic disease, the crucial discovery that led to this expanded view was the immunogenicity of many, if not of all, tumors in syngeneic and autochthonous hosts. The qualitative or quantitative deviation from normal antigenicity which stamps tumor cells, including the frequent appearance of molecular configurations occurring on normal tissue only in embryonic or neonatal life, endows the transformed cells with at least a measure of foreignness, as entities that must elicit an active host response.

This response is at least in part immunologic but, it must be added immediately, not necessarily only so. It has become evident at this conference that an immunologic formulation of neoplasia must be developed with caution and only in the light of a balanced host-parasite relationship perspective. The appearance of new macromolecules on cancer cells or of macromolecules phenotypically deviant in ontogenic time or in tissue location raises the possibility of a wide variety of enzymatic and hormonal, in addition to immunologic, responses by the host. Moreover, the discovery of a tumor-specific or at least tumor-associated immunology in the late 1950's coincided with the growing recognition in other areas of immunology that immune responses may effect not only the destruction of target cells but also their enhanced development and that immunologic reactions elicited by extraneous antigens can spill over specifically or indirectly into attacks on host constituents.

Simultaneous with the nascent awareness of the intricacy of the immunologic compartment of host defenses and of the diverse consequences of an immune response, the doctrine of specificity in disease and in immunologic relationships demanded modification (4, 5). The existence of cross-reactive antigens widely distributed in nature came to be recognized as a common occurrence rather than as an exception, and numerous microbial and nonmicrobial agents were discovered to have the capacity of modulating specific immunologic responsiveness nonspecifically (6-10). Clearly, then, immunologic responses against cancer cells are highly complex, both in their nature and in their consequences. The specificity of their elicitation and manifestation is not a simple matter, and it is probable that they are only part of the host's armament against neoplastic challenge. The theory of immune surveillance against progressive neoplasia remains a useful concept, but it and its many implications must be subsumed within the larger framework of the dynamic interactions of host and parasites.

THE HOST-PARASITE VIEW OF NEOPLASIA TODAY: DANGERS OF DISTORTION

The view of neoplasia as a dynamic association between host and neoplastic parasite has finally been accepted.

Much attention is now focused on the host component, and immunologic parameters of this association are in the limelight. Their preeminence today is hardly surprising. We have at our disposal new means of analysis, new questions, and new hopes of clinical intervention. Major inroads were made toward the control of infectious diseases even before the advent of antibiotics, and these victories were to a considerable extent based on immunologic manipulations. Similar success was to be expected of immunology for malignant diseases.

Yet for all the validity and empiric usefulness of the host-parasite concept of neoplasia, we can already see some of the misplacement and distortions of emphasis that are taking place within it. For one, the role of the host has been so strongly accentuated in the past several years that it overshadows the specific counteractions of which the neoplastic cell is often capable (11). Such counterattack can indeed be manifold; for instance, cancer cells have been reported capable of producing factors toxic to lymphoid tissue (12); of degrading host immunoglobulin molecules enzymatically, with the resultant production of antibody fragments devoid of cytotoxic properties but capable of effecting immunologic enhancement (13-15); of shedding into their environment antigenic cell membrane fragments (16) that may block T-cell receptors systemically and form around tumor foci gradients of material neutralizing immune elements (17, 18); of modulating their surface antigenic properties (11, 19, 20); and of other means of counterattack and escape from both immunologic and non-immunologic measures of control.

Moreover, the enthusiasm for immunology may be obscuring the existence of other modes of specific interaction between host and neoplastic cells. Warnings as to this overemphasis have already been sounded, and they should not be ignored. Klein has pointed out convincingly that immunologic defenses are likely to have evolved to a significant level of efficiency with regard only to those neoplastic diseases that pose a survival threat to species in nature, for example, Marek's disease of poultry, polyoma in wild rodents, and perhaps Burkitt's lymphoma of man (21, 22). A more radical stance has been taken by Prehn, who views immunologic reactions, both cellular and humoral, as often *responsible* for the excitation of progressive neoplastic growth (23, 24). We must now aim, therefore, for an evenhanded view of the extent and value of native immunologic capability by the hosts of neoplastic cells, and of the possibilities of immunologic intervention from without in those cases of neoplasia that do not represent natural threats to species survival (21, 22).

In addition to focusing more accurately on the host component of the host-parasite relationship of neoplasia, we must now carefully examine the importance of environmental parameters. These include not only the elements impinging on the interactants from the external milieu of a tumor-bearing host but also those of intrinsic origin, i.e., the internal tissue environment as it is shaped by genetic potential, sex, age, and the physical and psychologic responses to extrinsic stimuli. "Environment" subsumes the totality of all these variables interacting with each other and composing the continuously changing stage on which a biologic event is played out. It was pointed out forcibly by Schneider in the 1950's that environmental factors favoring the normal growth and reproductive capabilities of a species are not necessarily those that make for the best ability to resist microbial infections (25, 26). The same consideration most likely applies to resistance against neoplastic cells as

well, and it would seem probable that the determinants of optimum growth of transformed cells *in vitro* are not necessarily identical with those that best enable neoplastic cells to overcome the defenses of a reactive host.

Our goal must be, therefore, to arrive at a balanced formulation of neoplasia as a host-parasite relationship, as a contest for survival of opponents each of which is capable of defense and counterdefense and whose behavior in confrontation with one another is specifically influenced by the matrix of ambient and innate environment. The acceptance of a biologically valid conception of cancer can already be seen as more than intellectual nicety. It suggests at once new experimental pathways and emphases, and a receptivity to new interpretations of data. Attempts at insight into the particular problem before us today, the mechanisms of spontaneous regression of cancer, must clearly be linked to the wider question of the nature of the host-parasite association in malignant disease, and it would be appropriate, therefore, to devote the time remaining to a brief look at a few of the immediate and concrete implications for cancer research of the symbiosis conception of neoplasia (27).

CURRENT IMPLICATIONS FOR CANCER RESEARCH OF A BALANCED HOST-PARASITE VIEW OF NEOPLASIA

Neoplastic Potential of the Transformed Cell

It has long been recognized that clones of transformed cells vary widely in their ability to grow, invade, and metastasize and in their relative dependence on the physiologic ambience of the host. The nature of the parameters that delimit neoplastic potential remain largely obscure, however. It is suspected today that reactions of the host participate in the characterization of the neoplastic behavior of transformed cells, and further study of this participation will undoubtedly prove useful. At the same time, there must be a reemphasis on intrinsic properties of the parasite which may have a function. Among the possibilities of further parasite-focused exploration, the following may be cited.

In at least several models of neoplasia, distinct stages of neoplastic potency have been described. The most extensively studied example is that of mammary neoplasia in the mouse. The work of DeOme and Nandi (28), confirmed by other workers, has shown that there is a stepwise progression in the neoplastic capacity of mammary parenchyma of mice infected with mammary tumor viruses and that between the poles of normalcy and full neoplasia there lies a well-demarcated intermediate stage. Definition of the preneoplastic (or in Prehn's terminology, premalignant) condition and its special properties may be of salient significance to an understanding of the dynamics of host-tumor interaction, of the conditions of flagrant neoplasia, and of spontaneous regression. It has been found, for example, that at certain stages in mammary neoplasia of mice, the already transformed cells remain dependent on intimate spatial association with normal mammary tissue (29). Could disruptive influences on such association be defined, and to what extent might they be responsible for the "spontaneous" disappearance of various tumors consisting entirely or mainly of cells that have not yet reached a critical degree of independence on intimate contact with normal cells? What are the human analogies to the pre-neoplastic condition of murine mammary tissue? Are the various instances of human carcinoma *in situ* cases in point? To what extent do host immunologic and nonimmunologic responses and unique environmental circumstances

fix the preneoplastic stage and restrict further progression, and to what extent does preneoplasia represent intrinsic limitations of the transformation from normal? A great many additional questions can profitably be asked here.

It has been suggested (30) that in some instances, transformed variants are distinguished from their normal counterparts by the ability to continue growth and division after many repeated passages in vivo and in vitro; the normal cells, in contrast, have built-in restrictions as to the number of mitotic events of which the clone is capable. Could it not be that in some instances cell populations, possessing many of the characteristics of neoplastic growth and even of malignant behavior, nonetheless remain subject to a restricted mitotic capacity? In that event, spontaneous regression of a tumor would represent exhaustion of an intrinsic facility for growth. Can host responses have a bearing on such mitotic restriction of transformed cell populations? One possibility could be the establishment of subclones with lesser limitations as an active selective process, in which such cells are also distinguished by properties making for resistance to host attack. Another possibility could be the reverse event, in which variants with restricted mitotic ability would be selected for a resistance characteristic, only to disappear spontaneously at the term of their growth potential.

Active Destruction of Neoplastic Cells by Nonimmunologic Mechanisms

We have already alluded to the danger that the current enthusiasm for tumor immunology may bring an exaggeration of the role played by immunologic reactions in the overall defense system against neoplasia and a de-emphasis of other mechanisms. Many possible modes of active host resistance which can be envisaged have so far not even been subjected to casual examination. Only one example need be mentioned. It has been reported that spontaneous regression of cancer frequently follows an episode of acute microbial disease (31). The instinctive tendency in the present climate of opinion is to ascribe to the bacterial invaders nonspecific stimulatory effects on immunologic and resistance capacity (4, 8, 10), which leads to a more effective host response to the tumor. A variant immunologic explanation, which must be entertained, is that contact with microbial antigens may hyperimmunize the organisms specifically against the tumor cells, a common antigenicity between microbial moieties and a variety of neoplasms having now been demonstrated (32-34). There is, however, a possible nonimmunologic consequence of microbial infection that deserves consideration: Host tissue lytic enzymes, the production of which is stimulated by the microbial substrates, could perhaps also attack tumor cell substrates where these share common macromolecular configurations, as is suggested by the antigenic cross-reactivity between bacterial and neoplastic cells. Alternatively, perhaps bacterial enzymes could directly attack the transformed cells, and this may be the case as well for host enzymes without reference to any stimulation of their synthesis by cross-reactive bacterial determinants.

Other positive consequences for resistance not directly immunologic in nature can be imagined to accrue from bacterial infection. At least some types, or stages, of inflammatory processes directed at the microbial invader may be highly inimical to tumor cells as "innocent bystanders" (35). The possibility exists that some neoplastic cells may offer particularly favorable media for microbial growth, and infecting microorganisms may thus localize

in the vicinity of neoplastic foci and place them in jeopardy of injury from inflammatory reactions. It has been claimed by some investigators that administration of nonspecific immunologic modulators of bacterial origin must be directed into a tumor focus if a strong therapeutic effect is to be obtained (36). Although the generality of this requisite for intralesional administration is very doubtful (37-40), it may well be that in those cases in which it is necessary, intimate contact between tumor cells and the bacterial agent may be required to trigger an inflammatory bystander effect.

Environmental Determinants

Only a few examples need be given to stress the decisive influence that intrinsic and extrinsic environmental parameters may exert on the course of a neoplastic process.

Immunization of older organisms with tumor-associated antigens may fail to induce a significant increment in resistance to neoplastic cells unless the base line of immunologic capacity, low as a function of age, is first lifted by nonspecific immunostimulation (4, 41). Thus immunoprophylaxis and immunotherapy of cancer may require a combined nonspecific and specific excitation. On the other hand, nonspecific potentiation of immunologic function may also shift responsiveness in undesired directions, the production of noncytotoxic and blocking immune elements. This may be especially true in younger animals, in which humoral antibody responsiveness is still at a peak. Attempts at tumor immunization must thus take into consideration the native state of immunologic ability of the individual organism and the directions that immunopotential are likely to take. It appears mandatory for further development of the area of nonspecific immunologic intervention that agents be developed with predictable modulating effects on the immunologic apparatus of patients in a given age range (42, 43).

Another factor linked to host age relates to the nature of tumor-associated antigens. In a wide variety of cases, these have been found to be constituents appearing normally in fetal life and largely disappearing from normal tissues shortly after birth. Where such fetal antigens represent the obligatory targets of host immune attack, a question that immediately presents itself is: To what extent is the host tolerant to molecular constituents that appeared at earlier stages in his history? The ability of adult animals to respond to autochthonous primordial antigens has been demonstrated directly in some instances (44), and immunologic reactivity has been noted against many tumor cells, at least some of whose surface antigens are fetal. Nonetheless, it is quite possible that the organism becomes more capable, or differently capable, of reacting to such antigens as the time span between their moment of expression and the test for immunologic responsiveness increases; immunization of young animals with tumor cells characterized by embryonic antigens may thus yield limited results unless a state of partial unresponsiveness to them is first broken. So far, however, the factors of host age and innate immunologic strength are given little weight in the design of animal experiments.

Another example of an environmental determinant is that the usual laboratory test animals used for tumor immunology experiments are neither germfree nor specific pathogen free. Although the argument may be advanced that such "ordinary" animals bear a closer resemblance to the human tumor host than do organisms with a very restricted or wholly absent microbial flora (39), the effects

of extrinsic immunomodulation by agents of microbial origin may fluctuate markedly with variations in endemic infection, albeit of subclinical nature, and may be obscured entirely. For that matter, some endemic infections could conceivably result in states of antigenic competition with regard to tumor antigens, which in many instances are relatively weak immunogens at the start. It might be profitable, therefore, to define the microbial flora of the host in critical immunization experiments, i.e., in both situations, when immune stimulation is specific and when it is nonspecific.

Much attention needs to be given to the microenvironment of the tissues in which the confrontation between newly arisen and newly settled neoplastic cells and the host takes place. The vertebrate organism cannot be considered as a single immunologic unit but rather must be viewed as an assembly of overlapping subcompartments, each one forming a distinct immunologic microcosm, in controlled rather than free-flowing communication with each other. It occurs not infrequently that spontaneous and experimentally induced tumors grow progressively and even rapidly, but with little tendency to metastasize, and are unable to take hold upon transfer to a syngeneic host. A recent case in point are the UV-induced sarcomas studied by Margaret Kripke (personal communication, 1974). These appear to be highly antigenic neoplasms, not transplantable to normal syngeneic mice but capable of progressive and ultimately fatal development in the host of origin. Local failure to contain tumors despite coexistent states of systemic resistance may be immunologic or nonimmunologic; the occasional success of a host with good concomitant immunity to overcome a focal deficiency would be expressed as unanticipated spontaneous regression.

Related to this consideration of tissue microenvironment is the problem of preferential localization of systemically disseminated tumor cells and of privileged holding sites in which such cells can develop progressively while failing to establish themselves elsewhere (45). Although differential tissue susceptibility to neoplastic growth is one of the best documented phenomena of clinical oncology, relatively little attention has as yet been focused on the immunologic and other determinants of the geography of neoplasia in the individual. Here definitive progress may be expected if this geographic distribution is reexamined in the light of host-parasite relationship dynamics.

Technical Considerations

In the area of infectious diseases, parasitic microorganisms often behave very differently when freshly isolated from their host than after even one or two *in vitro* transfers, or even after passage in an animal other than the natural reservoir species. That this probably holds true for neoplastic cell parasites as well has been suggested by a number of investigators. I shall cite only two recently described cases in point: YAC Moloney lymphoma cells change markedly in their sensitivity to antibody-mediated cytotoxicity upon explantation from a murine host to tissue culture (Witz IP, Klein E: Personal communication, 1974). The tumor cells may become more or less susceptible, depending on their initial *in vivo* sensitivity, within 60 minutes after placement into tissue culture. A similar observation by the Klein group pertains to changes in the sensitivity of cells of a 3-methylcholanthrene-induced sarcoma to "natural" cytotoxic antibodies in normal rabbit serum; the antigen(s) seemingly responsible for the susceptibility of the cells sheds very rapidly into the culture medium.

Indeed, a variable susceptibility to immune attack by tumor cells propagated *in vivo* and *in vitro* has been documented in some instances (46). The selection upon repeated *in vivo* passage of subpopulations of tumor cells that do not express, or express to a lesser extent, characteristics—antigenic or other (47)—allowing susceptibility to immune attack would not be an unexpected phenomenon. It must be noted, however, that failure to demonstrate such a selection has been reported with some neoplasms (48). Other differences between neoplastic cells growing *in vivo* and cultured *in vitro* must be anticipated. Nonetheless, much work in tumor immunology, and in tumor biology in general, continues to be conducted with neoplastic cells long carried *in vitro* or *in vivo*, with little attention to the probability that the results coming from such investigations may be highly artifactual.

Not only may neoplastic cells display variant characteristics depending on their most recent growth environment, including traits making for greater or lesser refractoriness to host attack, but also the conditions of *in vitro* assessments of resistance are radically different from those pertinent *in vivo*. The *in vitro* test system can attempt to minimize artificial alterations in the nature of the interacting partners. It can never be assumed, however, that major deviations from the natural have not in fact occurred and that all the host factors that react with the target cell and that can influence each other's activities remain sufficiently intact to fulfill their roles. At best, we can hope to obtain from *in vitro* analyses approximations of the capacities of isolated components of the resistance apparatus; these impressions may be highly misleading. Thus, for instance, certain types of tumors exhibit broad antigenic cross-reactivity when tested *in vitro* but not *in vivo* (49), perhaps because of antigen blocking of lymphocyte receptor sites for the common antigen (often embryonic in nature?). Another eventuality is that the cytotoxic activity of isolated lymphoid cells *in vitro* may fail to reflect corresponding levels of resistance by the intact host at that moment in the development of the neoplastic process (50, 51).

The greater ease of characterizing and quantifying aspects of host defense by *in vitro* study lent considerable impetus to this approach during the past 10 years and to the development of elegant methods for the measurement of cell- and antibody-mediated cytotoxicity in tissue culture. It has now become evident, however, that the results of all *in vitro* experiments must be interpreted with much caution; opinion is clearly swinging back to a reliance on *in vivo* observations for critical information. This shift in attitude is indeed unavoidable: The visualization of neoplasia as a host-parasite relationship cannot permit dependence on *in vitro* analyses with their unavoidable reduction and fragmentation of biologic reality.

Immunologic Aspects of Tumor Resistance

The reservations already expressed against a facile exaggeration of the place of immunologic reactions in host-tumor relationships are not, of course, intended to deprecate tumor immunology. Quite to the contrary! The plea for a balanced stance by the student of neoplasia is incumbent today, but without negating the current expectation that immunology will indeed contribute significantly to our understanding of progressive neoplasia and to its eventual control.

The various possibilities of immunologic intervention in cancer—prophylactic, diagnostic, and therapeutic—have been reviewed recently (11). On this occasion, I wish only

to illustrate how the host-parasite relationship conception of neoplasia must influence immunologic directions of investigation as well.

First, there has been a tendency to conceive failure of immune surveillance against progressive neoplasia in very general terms. This may be incorrect. Deficiency of immunologic capacity may be very specific. Individual strain- and species-associated susceptibility may accrue from genetic restrictions of immunologic responsiveness toward a very limited number of tumor-associated antigens, with no indication of broad immunologic dyscrasia; such focused incapacity has been incriminated in exaggerated susceptibility to microbial pathogens as well. It has been suggested, for instance, that certain normal tissue antigens of some individuals may be cross-reactive with the surface antigens of some microorganisms, thereby compromising immunologic reactivity against them (52, 53). The demonstration of shared antigens between some tumor cells and a variety of bacteria (32-34), the fetal nature of many tumor-associated antigens, and the suggestion that many neoplastic cells prominently express surface molecular configurations ubiquitous in normal tissue as "basic" cell membrane entities (54) all point to the likelihood of highly selective immunologic failure vis-à-vis transformed clones. We may, accordingly, have to focus our future efforts on an overcoming of specific deficiencies as much as on a general potentiation of the immunologic mechanism. There are a number of possibilities for breaking states of specific immunologic unresponsiveness, such as immunization and hyperimmunization with chemically modified immunogens and treatment with modulators of immunologic responsiveness that have the capacity of strengthening and directing immunologic reactivity to very weak immunogens (42, 43) and of preventing or reversing states of specific tolerance (4, 8, 55).

Second, we have already alluded to the likelihood that the seeming failure of host defenses against neoplastic cells may frequently result from active evasion by the transformed cells rather than from an intrinsic incapacity of the host to react. Only a few specific instances of such avoidance mechanisms need be discussed as illustration.

Having long stood in disrepute as agents of immunologic enhancement or, at best, as ineffective in the attack on tumor cells, free antibodies have come to be recognized recently as an important component in the immune reactions mounted against cancer cells. They appear to be active by themselves in mediating resistance against some neoplastic cells (56, 57); they are suspected to be the factors responsible for the "arming" of macrophages (58), which are increasingly thought to have a large part in the destruction of neoplastic variants (10, 59); and they have been characterized as the bridges between tumor target cells and a variety of apparently distinct macrophage-like and lymphocyte-like cells, collectively termed K cells, which lack specific receptors for target cells but effect their destruction by virtue of the antibody linkage (60). Such K cells are today thought to be the cells largely responsible for the destruction of certain types of tumors. Moreover, it is becoming increasingly apparent that the final expression of immunologic reactivity in a given direction reflects the net results of complex and multifaceted interactions, both central and peripheral, between humoral and cellular immune components; thus antibodies most likely have a significant indirect as well as direct function in immunologic tumor destruction. The findings that tumor cells specifically adsorb and sequester significant amounts of

humoral antibodies (61) and that they can actively degrade even cytotoxic antibodies into Fab fragments with enhancing properties may represent a central mechanism of neoplastic cell escape.

Another analogy to host-parasite associations in the area of infectious disease may be made with regard to antibodies. It has long been shown that the attachment of antibodies to virulent bacteria not only facilitates their phagocytosis but also can protect the engulfing phagocyte from their pathogenic action and aid their intraphagocytic destruction (62). Macrophages come into play against tumor cells both as cells capable of direct contact cytotoxicity when they are armed or "excited" (58) and as actively phagocytic entities. It would appear, therefore, that the role of antibodies deserves attention in determination of the outcome of phagocyte-tumor cell interaction at several levels.

A second major escape route of neoplastic cells from host attack may derive, as indicated above, from the shedding of surface antigenic components. Such peeling off may not be at all qualitatively unique to transformed cells, and it has been suggested that tolerance to self constituents may depend on a continuous blocking by shed antigens of the receptors of normally present lymphocytes directed against self markers (63). Neoplastic cells may thus preempt a normal and essential homeostatic mechanism in order to escape immune destruction. The pendulum of opinion has indeed now swung to the view that enhancement of tumor growth may, in fact, be due more to antigen blocking of specific lymphoid cells than to antibody blocking at the level of the targets.

A number of questions arise from this reorientation of thinking as to the mode of action of interference with immunologic control: What are the relative kinetics of cell membrane shedding by normal and transformed cells? What function does such shedding have in systemic and in focal blocking of immunologic reactivity? Is there any correlation between malignant potential and virulence of neoplastic cells and their ability to release membrane constituents? What are the different biologic consequences of the shedding of *particulate* and of *soluble* surface antigens? Can the shedding of surface components be inhibited by extrinsic agents and perhaps even by free antibody? How complete must effector cell blocking by tumor antigen be to interfere categorically with the ability of lymphoid-reticuloendothelial system cells to effect intimacy with their targets and to destroy them? Is there, perhaps, a variable order of affinities and avidities of receptor-antigen binding that determines differentially the biologic consequences? Is it conceivable that a residual degree of attachment of partially blocked effector cells to target cells may prevent cytotoxic action and actually lead to growth stimulation of the incompletely embraced neoplastic targets? Would it be possible to achieve therapeutic results by taking lymphoid cells from a tumor host, removing the blocking antigens, and returning the then activated cells? Perhaps the only immediate usefulness of these questions is that they are all subject to experimental examination; in light of the fact that definitive clinical intervention in malignant diseases by immunologic means has so far largely eluded us, even this is an advantage.

Some attention has recently been given to a more generalized inhibition of immunologic capacity resulting from the production in the host of "normal immunoregulatory proteins" (64), and the possibility has been advanced that such substances may be produced not only by

normal host tissues but also by neoplastic cells (65). Can the liberation of such factors be controlled or their activity neutralized? It is evident that a major aim of ongoing investigations in tumor immunology should be the understanding of the means of neoplastic cell escape and the elucidation of possibilities of closing the escape routes.

CONCLUDING COMMENTS

The neoplastic and pathogenetic behavior of transformed cells is not a static attribute but rather the expression of a highly dynamic concatenation of events in which host and environmental variables have large roles. We must think of potentialities—potentials of neoplasia and of malignancy and of defense realized in the fluid interactions of host, parasite, and environment—rather than of immutable traits, if we are to encompass the condition of neoplasia in a biologically realistic framework.

Perhaps the eradication of neoplastic cells in a cancer patient is a wholly elusive, and even unnecessary, therapeutic goal. Here, again, there may be a useful reference to host-parasite relationships involving the microbial world. The health of individuals and the survival of species are certainly not predicated on life in a sterile environment but rather on the attainment of a *modus vivendi* with large numbers of different potential pathogens. Only when the "normal" equilibrium of coexistence with microorganisms is markedly or repeatedly disturbed do we consider ourselves seriously threatened. Perhaps the situation is not dissimilar vis-à-vis neoplastic parasites. They, too, may be ubiquitous in our internal environment, arising constantly and, under "normal" conditions, prevented from progressive growth. Indeed, the dissemination of tumor cells and their long persistence in the tissues of surgically cured cancer patients are perhaps more the rule than the exception, as is the presence, especially in older individuals, of islands of neoplastic tissue that remain sufficiently insular to permit what we consider a normal life-span and normal health. If our focused therapeutic goal were to be the more modest one of containment of neoplastic variants, we might perhaps come closer to attaining a measure of success, that is, a tolerable armistice with cancer cells, than if we set our sights at their extirpation or at effecting *in vivo* a complete reversal of their neoplastic characteristics (66).

Attenuated tubercle bacilli such as the BCG strain behave like fully virulent organisms in a silicotic host (67). Tumors not transplantable in normal syngeneic hosts often grow rapidly, invade, and metastasize in animals irradiated or otherwise subjected to insults to their immunologic apparatus, including treatment with cancer chemotherapeutic agents. It may not be unreasonable to hope that manipulations of host and environment in the opposite direction could cause malignant populations of neoplastic cells to behave like attenuated pathogens.

This discussion began with the theme that an understanding of the exceptional occurrence of spontaneous regression of cancer may lead to a more profound understanding of the basic dynamics of neoplasia. I conclude with the thought that, in turn, new insights into these dynamics might enable us to intervene in malignant pathologies of man in such a manner as to make the regression of established disease a less rare event.

REFERENCES

(1) WEISS DW: Immunological parameters of the host-parasite relationship in neoplasia. *Ann NY Acad Sci* 164:421-448, 1969

- (2) —: Immunological parameters of host-tumor relationships: Spontaneous mammary neoplasia of the inbred mouse as a model. *Cancer Res* 29:2368-2373, 1969
- (3) —: Perspectives of host-tumor relationships. Preface and Introduction, *In Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.). New York, Academic Press, 1971, pp 1-6
- (4) WEISS DW, YASHPHE DJ: Nonspecific stimulation of antimicrobial and antitumor resistance and of immunological responsiveness by the MER fraction of tubercle bacilli. *In Dynamic Aspects of Host-Parasite Relationships* (Zuckerman A, Weiss DW, eds.), vol 1. New York, Academic Press, 1973, pp 163-223
- (5) WEISS DW: Current aspects of tumor immunology. Preface and Introduction, *In Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 2. New York, Academic Press, 1973, pp 1-12
- (6) SHILO M: Nonspecific resistance to infections, *Ann Rev Microbiol* 13:255-278, 1959
- (7) YASHPHE DJ: Immunological factors in nonspecific stimulation of host resistance to syngeneic tumors. A review. *In Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.). New York, Academic Press, 1971, pp 90-107
- (8) WEISS DW: Nonspecific stimulation and modulation of the immune response and of states of resistance by the methanol-extraction residue fraction of tubercle bacilli. *Natl Cancer Inst Monogr* 35:157-171, 1972
- (9) WOLSTENHOLME GE, KNIGHT J, eds.: Immunopotential. *Proceedings. CIBA Foundation Symposium No. 18.* Amsterdam, Elsevier, 1973
- (10) LEDERER E, ADAM A, CIORBARU R, et al: Cell walls of mycobacteria and related organisms. Chemistry and immunostimulant properties. *Mol Cell Biochem*, In press
- (11) WEISS DW: Immunological intervention in neoplasia. *In The Role of Immunological Factors in Viral and Oncogenic Processes* (Beers RF Jr, Tilghman RC, Bassett EG, eds.). (The Johns Hopkins Med J suppl No. 3.) Baltimore, The Johns Hopkins Univ Press, 1974, pp 131-169
- (12) PIKOVSKY MA: Studies on different stages in host-tumor relationship. 1. Differences in the behavior *in vitro* of ascites cells and lymphocytes derived from mice in early and later stages of tumor growth. *Isr J Med Sci* 9:136-144, 1973
- (13) WITZ IP, LEVY HJ, KEISARI Y, et al: Tumor-bound immunoglobulins: Their possible role in circumventing antitumor immunity. *In The Role of Immunological Factors in Viral and Oncogenic Processes* (Beers RF Jr, Tilghman RC, Bassett EG, eds.). (The Johns Hopkins Med J suppl No. 3.) Baltimore, The Johns Hopkins Univ Press, 1974, pp 289-300
- (14) KEISARI Y, WITZ IP: Degradation of xenogeneic cytotoxic antitumor antibodies by tumor-derived lysosomal enzymes and the generation of products blocking humoral cytotoxicity. *Behring Inst Mitt* 56:19-27, 1975
- (15) DAUPHINEE MJ, TALAL N, WITZ IP: Generation of non-complement fixing, blocking factors by lysosomal extract treatment of cytotoxic antitumor antibodies. *J Immunol* 113:948-953, 1974
- (16) BEN-SASSON Z, WEISS DW, DOLJANSKI F: Specific binding of factor(s) released by Rous sarcoma virus-transformed cells to splenocytes of chickens with Rous sarcomas. *J Natl Cancer Inst* 52:405-412, 1974
- (17) CURRIE GA, BASHAM C: Serum-mediated inhibition of the immunological reactions of the patient to his own tumour: A possible role for circulating antigen. *Br J Cancer* 26:427-438, 1972
- (18) MORE R, YRON I, BEN-SASSON Z, et al: *In vitro* studies on cell-mediated cytotoxicity by means of a terminal labeling technique. *Cell Immunol* 15:382-391, 1975
- (19) CIKES M, FRIBERG S JR, KLEIN G: Progressive loss of H-2 antigens with concomitant increase of cell-surface antigen(s) determined by Moloney leukemia virus in cultured murine lymphomas. *J Natl Cancer Inst* 50:347-362, 1973
- (20) KILLANDER D, YEFENOF E, KLEIN E: Antibody-induced redistribution of surface antigen is independent of the amount of antigen and the cell cycle position. *Exp Cell Res* 89:413-415, 1974
- (21) KLEIN G: Immunological surveillance. *In Proceedings of the 26th Annual Symposium on Fundamental Cancer Research* (Cumley RW, ed.). Houston, M. D. Anderson Hospital and Tumor Institute. In press
- (22) —: Herpesviruses and oncogenesis. *Proc Natl Acad Sci USA* 69:1056-1064, 1972
- (23) PREHN RT, LAPPE MA: An immunostimulation theory of tumor development, *Transplant Rev* 7:26-54, 1971

- (24) —: The immune reaction as a stimulator of tumor growth. *Science* 176:170-171, 1972
- (25) SCHNEIDER HA: Nutrition and resistance—susceptibility to infection. *Am J Trop Med* 31:174-182, 1951
- (26) —: Nutrition and resistance to infection. *Borden's Rev Nutr Res* XIV(2):17-32, 1953
- (27) WEISS DW, ZUCKERMAN A: Preface and Introduction. In *Dynamic Aspects of Host-Parasite Relationships* (Zuckerman A, Weiss DW, eds.), vol 1. New York, Academic Press, 1973, pp vii-xii
- (28) DEOME KB, NANDI S: The mammary-tumor system in mice, a brief review. In *Viruses Inducing Cancer* (Burdette WJ, ed.). Salt Lake City, Univ Utah Press, 1966, pp 127-137
- (29) SLEMMER G: Host response to premalignant mammary tissues. *Natl Cancer Inst Monogr* 35:57-71, 1972
- (30) DANIEL CW, DEOME KB, YOUNG JT, et al: The in vivo life span of normal and preneoplastic mouse mammary glands: A serial transplantation study. *Proc Natl Acad Sci USA* 61:53-60, 1968
- (31) NAUTS HC: The apparently beneficial effects of bacterial infections on host resistance to cancer (Monogr 8, NY Cancer Inst Publ). New York, 1969
- (32) BORSOS T, RAPP HJ: Antigenic relationship between *Mycobacterium bovis* (BCG) and a guinea pig hepatoma. *J Natl Cancer Inst* 51:1085-1086, 1973
- (33) MINDEN P, McCLATCHY JK, WAINBERG M, et al: Shared antigens between *Mycobacterium bovis* (BCG) and neoplastic cells. *J Natl Cancer Inst* 53:1325-1331, 1974
- (34) BUCANA C, HANNA MG JR: Immunoelectronmicroscopic analysis of surface antigens common to *Mycobacterium bovis* (BCG) and tumor cells. *J Natl Cancer Inst* 53:1313-1323, 1974
- (35) PREHN RT: Destruction of tumor as an "innocent bystander" in an immune response specifically directed against nontumor antigens. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 2. New York, Academic Press, 1973, pp 171-175
- (36) RAPP HJ: A guinea pig model for tumor immunology. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 2. New York, Academic Press, 1973, pp 162-170
- (37) YRON I, WEISS DW, ROBINSON E, et al: Immunotherapeutic studies in mice with the methanol-extraction residue (MER) fraction of BCG: Solid tumors. *Natl Cancer Inst Monogr* 39:33-54, 1973
- (38) HARAN-GHERA N, WEISS DW: Effect of treatment of C57BL/6 mice with the methanol extraction residue fraction of BCG on leukemogenesis by the radiation leukemia virus. *J Natl Cancer Inst* 50:229-234, 1973
- (39) YRON I, COHEN D, ROBINSON E, et al: Effects of MER and therapeutic irradiation against established isografts and simulated local recurrence of mammary carcinomas. *Cancer Res* 35:1779-1790, 1975
- (40) COHEN D, YRON I, HABER M, et al: Effect of treatment with the MER tubercle bacilli fraction on the survival of mice carrying mammary tumour isografts: Injection of MER at the tumour site or at a distal local location. *Br J Cancer*. In press
- (41) PASS E, JACOBS DM, WEISS DW: Modulation of the immune response by a methanol-insoluble fraction of attenuated tubercle bacilli (BCG). III. Stimulation of antibody synthesis to a soluble protein, BGG. Submitted for publication
- (42) BEN-EFRAIM S, CONSTANTINI-SOUROJON M, WEISS DW: Potentiation and modulation of the immune response of guinea pigs to poorly immunogenic protein-hapten conjugates by pretreatment with the MER fraction of attenuated tubercle bacilli. *Cell Immunol* 7:370-379, 1973
- (43) BEN-EFRAIM S, TEITELBAUM R, OPHIR R, et al: Nonspecific modulation of immunological responsiveness in guinea pigs and mice by the MER mycobacterial fraction: Influence of conditions of MER treatment and specific immunization, and effect of MER on early stages in the immune response. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 3. New York, Academic Press. In press
- (44) TRIPLETT EL: On the mechanism of immunologic self recognition. *J Immunol* 89:505-510, 1962
- (45) WEISS DW: Summary: Antigens in preneoplastic tissue during tumorigenesis. *Natl Cancer Inst Monogr* 35:89-93, 1972
- (46) FENYÖ EM, KLEIN E, KLEIN G, et al: Selection of an immunoresistant Moloney lymphoma subline with decreased concentration of tumor-specific surface antigens. *J Natl Cancer Inst* 40:69-89, 1968
- (47) KLEIN G, KLEIN E, HAUGHTON G: Variation of antigenic characteristics between different mouse lymphomas induced by the Moloney virus. *J Natl Cancer Inst* 36:607-621, 1966
- (48) SJÖGREN HO: Studies on specific transplantation resistance to polyoma-virus-induced tumors. IV. Stability of the polyoma cell antigen. *J Natl Cancer Inst* 32:661-666, 1964
- (49) BALDWIN RW, GLAVES D, VOSE BM: Embryonic antigen expression in chemically induced rat hepatomas and sarcomas. *Int J Cancer* 10:233-243, 1972
- (50) BARSKI G, JUNG KY, LE FRANÇOIS D, et al: Evolution of specific cell-bound immunity in hosts bearing solid tumors as related to tumor growth and treatment. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 3. New York, Academic Press. In press
- (51) BANSAL SC, SJÖGREN HO: Antitumor immune response and its manipulation in a tumor-bearing host. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 3. New York, Academic Press. In press
- (52) ROWLEY D, JENKIN CR: Antigenic cross-reaction between host and parasite as a possible cause of pathogenicity. *Nature* 193:151-154, 1962
- (53) JENKIN CR: An antigenic basis for virulence in strains of *Salmonella typhimurium*. *J Exp Med* 115:731-743, 1962
- (54) CASPARY EA, FIELD EJ: Specific lymphocyte sensitization in cancer: Is there a common antigen in human malignant neoplasia? *Br Med J* 2:613-617, 1971
- (55) PASS E, YASHPHE DJ: Stimulation of antibody synthesis to soluble bovine gamma globulin (BGG) by a methanol-extraction residue (MER) of BCG. *Isr J Med Sci* 7:609-610, 1971
- (56) MORTON DL: Humoral tumor immunity in man: Possible role in host defense against cancer. In *Membranes and Viruses in Immunopathology* (Day SB, Good RA, eds.). New York, Academic Press, 1972, pp 553-562
- (57) BLOOM ET, HILDEMAN WH: Mechanisms of tumor-specific enhancement versus resistance toward a methylcholanthrene-induced murine sarcoma. *Transplantation* 10:321-333, 1970
- (58) EVANS R, ALEXANDER P: Mechanism of immunologically specific killing of tumour cells by macrophages. *Nature* 236:168-170, 1972
- (59) NELSON DS: Immunity to infection, allograft immunity, and tumor immunity: Parallels and contrasts. *Transplant Rev* 19:226-254, 1974
- (60) FORMAN J, MÖLLER G: The effector cell in antibody-induced cell-mediated immunity. *Transplant Rev* 17:108-149, 1973
- (61) WITZ IP: The biological significance of tumor-bound immunoglobulins. *Curr Top Microbiol Immunol* 61:151-171, 1973
- (62) SUTER E, RAMSEIER H: Cellular reactions in infection. *Adv Immunol* 4:117-173, 1964
- (63) COHEN IR, WEKERLE H, FELDMAN M: The regulation of self-tolerance. Implications for immune surveillance against tumor cells. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 3. New York, Academic Press. In press
- (64) NELKEN D: Normal immunosuppressive protein (NIP). *J Immunol* 110:1161-1162, 1973
- (65) CLARK DA, NECHELES TF, NATHANSON L, et al: Apparent HL-A 5 deficiency in human malignant melanoma. II. HL-A 5 masking activity in sera of patients with progressive disease. In *Immunological Parameters of Host-Tumor Relationships* (Weiss DW, ed.), vol 3. New York, Academic Press. In press
- (66) INBAR M, RABINOWITZ Z, SACHS L: The formation of variants with a reversion of properties of transformed cells. III. Reversion of the structure of the cell surface membrane. *Int J Cancer* 4:690-696, 1969
- (67) VORWALD AJ, DWORSKI M, PRATT PC, et al: BCG vaccination in silicosis. *Am Rev Tuberc* 62:455-474, 1950

IV.

SPECULATIONS, HYPOTHESES, AND THEORETICAL CONSIDERATIONS



Metastatic Spread and "Escape" From the Immune Defenses of the Host¹

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SUMMARY—The effectiveness of the host's immune reaction against primary and disseminated tumors depends not only on the magnitude of the host's immune response, such as the number of cytotoxic cells and antibody molecules produced, but also on the capacity of tumor cells to evade destruction. The latter process which has been termed "escape" depends on several factors including intrinsic properties of the tumor cell. In some experimental systems, it was shown that the capacity of disseminated tumor cells to give rise to distant metastases is in part determined by the efficiency of escape. Tumors which in vivo appear to be nonimmunogenic may still carry tumor-specific antigens to which the host responds by making cytotoxic mononuclear cells, but these fail to kill because escape is effective.—*Natl Cancer Inst Monogr* 44: 125-129, 1976.

Sir Michael Woodruff coined the word "escape" for the phenomenon that permits tumors to grow, metastasize, and kill in spite of the fact that the host reacts against an antigen [usually referred to as tumor-specific transplantation antigen(s) (TSTA)] on the surface of the tumor cells. The antigen induces cytotoxic mononuclear cells and antibody, which in vitro can be shown to kill cells derived from the tumor. Escape is not unique to tumors; it contributes to the "success" of many microbial diseases. A positive tuberculin reaction does not signify that the disease is contained, and the patient with tuberculosis fails to mount an immune reaction only in the very terminal stages of the disease. Indeed, there are striking similarities between the persistence of infectious diseases and cancer, and the tumor immunologist can learn much from students of infectious disease, particularly from work done some 40 years ago, before effective chemotherapeutic agents for the control of infectious disease began to be discovered.

Escape has to be considered at two levels of tumor growth; once the tumor has become clinically detectable and contains in excess of 10^9 malignant cells, its growth despite an active host response may be explicable in purely quantitative terms; that is, the rate at which new tumor cells appear is greater than the production of immune effector arms. The more subtle and perhaps more important problem is that small tumor masses in the early stage of tumor growth are resistant to attack by the host; the most important problem of all is that isolated cells distant from the primary tumor (which can be dealt with by surgery or radiotherapy) persist and eventually give rise to metastases. Escape of relatively small tumor masses is a multifactorial phenomenon (1). Some biologic mechanisms considered relevant to escape of antigenic tumor are listed below:

- A. Not of general importance
 1. Specific tolerance to tumor-specific antigen.

2. Generalized immune defect (genetic, aging, drug-induced).
- B. Of general importance
 1. Interference by immunologically specific humoral factors that "inhibit" cytotoxic cells or "block" target cells.
 2. Anatomic factors (unequal distribution of various effector processes).
- C. Role remains to be established: Membrane antigen does not constitute target for cytotoxic attack.

FAILURE OF HOST TO RESPOND ADEQUATELY

Some years ago it was claimed that mice with leukemias and mammary carcinomas resulting from the vertical transmission of a virus (these were called "spontaneous" tumors because the animals were not subjected to a carcinogenic stimulus) had developed true tolerance to TSTA shown to be present on these tumor cells. More recent studies failed to sustain these claims, and animals bearing such tumors were shown to respond to the relevant TSTA. In man, evidence for the existence of TSTA relies almost entirely on the detection of an immunologically specific reaction of the host to the tumor; if there is true tolerance to the TSTA, then the tumor would be described as "non-antigenic." Also, some time ago it was suggested that tumor growth was associated with an immune defect in the host; this concept came from clinical studies that showed that some patients with advanced disease were immunologically anergic and unable to respond to many types of immunologic challenge. This immunologic anergy, however, is a consequence of advanced disease and not a cause, since the patients recovered a normal immunologic responsiveness after treatment that reduced the total tumor burden. This is not to say that immunosuppression does not facilitate the growth and spread of existing tumors.

INTERFERENCE BY HUMORAL FACTORS OF THE HOST

Mononuclear leukocytes from tumor bearers (both humans and experimental animals) have in many instances been cytotoxic in vitro to malignant cells from the tumor. This immunologically specific cytotoxic reaction of leukocytes is antagonized by the presence of serum from the tumor bearer. This interference by serum is, again, immunologically specific. Hellström and Hellström (2) claimed that this interfering factor had a specific affinity for the TSTA of the tumor cells and identified it as a "7S non-complement-binding" antibody that covered the target cells and shielded them from attack by cytotoxic mononuclear cells. In subsequent studies, however, Sjögren et al. (3) failed to isolate such blocking antibodies and claimed that the serum factor that specifically bound to the target cells and protected them against the cytotoxic cells was an antigen-antibody complex.

Work from our laboratory on both human (4) and experimental tumors (5, 6) showed that in the sera of tumor bearers, an immunologically specific factor combined not with the tumor cells but with the cyto-

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toxic mononuclear cells. There can be little doubt that the active principle in this inhibitor is soluble TSTA released from the tumor into the circulation. All available facts are consistent with the hypothesis that the prime mover in what has been called "the blocking reaction" of sera from tumor-bearers is, in fact, soluble tumor antigen. Under certain circumstances, antigen as part of an antigen-antibody complex can still interfere with the cell-mediated cytotoxic reaction in vitro. However, the active component of the complex is antigen, and antibody has no role. The fact that antibody has no part either in vivo or in vitro in protecting tumor cells against destruction by cytotoxic mononuclear cells was decisively demonstrated by Linna et al. (7), who found that bursectomy of birds, which renders them completely unable to form antibodies, does not interfere with the growth of tumors in such birds, nor does it affect the capacity of their sera to "block" the cytotoxicity of mononuclear cells from the blood. The nature of the factor(s) in sera of patients with advanced cancer and in experimental animals bearing large syngeneic tumors that antagonize host immune response in vitro is described as follows:

- A. Properties of serum inhibitor (circulating tumor-specific antigen)
 1. Inhibits cytotoxicity to tumor of mononuclear cells from sera of patients and rats with tumors.
 2. Combines with cytotoxic cells but not with tumor cells in the in vitro test system.
 3. Has molecular weight less than 100,000.
 4. Substances with similar properties can be isolated from urine.
 5. Substances with similar properties can be isolated from tumor membrane (man and rat).
 6. In rats, immunization with the soluble antigen (with adjuvant) from chemically induced sarcoma does not induce immunity.
- B. Experimental evidence for the presence of circulating TSTA's in serum
 1. Inhibition of cytotoxicity of mononuclear cells from blood or lymph nodes of tumor bearers: in man (4) and rat (8).
 2. Serology
 - a. Neutralization of syngeneic antisera obtained from rats after excision of tumor (5).
 - b. Radioimmunoassay using ^{125}I -labeled TSTA isolated from tumor by affinity chromatography (6).

Knowledge that antigen shed by growing tumors can assist in escape in vivo came from studies that showed that the nodes draining a growing tumor, while highly stimulated, did not function normally because the stimulated lymphocytes in the node were paralyzed by antigen released from the tumor and draining into the local node (9). Subsequent to these studies, the evidence listed above has accumulated, showing that the TSTA, which constitutes the point of attack by the host against the autochthonous tumor, is present in a soluble form in the body fluids of the tumor bearer. These soluble antigens are effective in providing escape because they combine with the effector arms (antibodies as well as specific cytotoxic cells) and thereby neutralize them. Moreover, in a soluble form the TSTA of the chemically induced tumors studied by us are very poor immunogens (at least in the absence of adjuvants) and do not stimulate the immune defenses of the host nearly so well as when they are presented as part of the tumor membrane.

It is interesting that also in bacterial disease, antigens released from the microorganisms into the circulation provide a potent escape mechanism. The virulence of different strains of *Pneumococcus* has been directly correlated with the release of antigen by the *Pneumococcus*. The virulent

pneumococci release so-called "soluble specific substance (triple S)" in large quantities, and this material combines with the antibody directed against the organism and thus allows the organism to proliferate unopposed. In cancer as well as in bacterial infections, the serum moves from antibody to antigen excess as the disease advances, and antigen-antibody complexes can usually be detected. As I shall show later, such antigen-antibody complexes in vivo do not facilitate escape, but only in carefully manipulated in vitro situations do they (by introducing antigen into the system) protect tumor cells against destruction by cytotoxic leukocytes.

ANATOMIC FACTORS

The body is not a suspension of cells in a test tube; highly effective anatomic barriers prevent the effector arms of the immune response from distributing themselves evenly throughout the body. Anatomic factors contribute to escape of tumors and of bacteria. The *Streptococcus* that causes puerperal fever of childbirth thrives in the uterus, which is highly disorganized, but does not grow elsewhere in the body. Bacterial endocarditis is another example of bacteria growing at one site (the heart valve) but not elsewhere. The effectiveness of antibody in protecting the vascular space and its ineffectiveness in protecting extravascular sites have been well demonstrated in experiments in which antibody was passively injected to protect animals against bacterial infection.

The role of anatomic factors in tumor immunity has been shown in a similar way, but it is perhaps most strikingly demonstrated by the phenomenon of concomitant immunity. It has been known for a long time that tumor-bearing animals are able to reject, to some extent, a further challenge of tumor if the cells are given at a site distant to the growing tumor. The extent of this concomitant immunity, however, varies from one challenge site to another, but the immunity is most powerfully expressed if the second challenge with tumor cells is given iv or ip (10). (See table 1). In some tumor systems, resistance to an iv challenge with tumor cells is as great in an animal bearing a subcutaneously growing tumor as in an animal that has been immunized by surgical excision of the growing tumor.

Evidence for the last mechanism listed above as possibly contributing to escape (i.e., membrane antigen does not constitute a target for cytotoxic attack) is only beginning to be observed. In many experimental tumors, the host produces antibodies and specifically allergic mononuclear cells directed against a component of the membrane of tumor cells, but these immune responses are unable to kill the tumor cells in vitro or in vivo. These tumor-associated antigens (which are usually of embryonic origin, although situated in the cell membrane of the tumor), do not constitute a target for cytotoxic attack (11, 12). It is possible that these membrane antigens are shed from the tumor cells before the effector arms of the immune response, which have combined with them, can kill the tumor cells.

IMMUNOLOGIC FACTORS IN THE CONTROL OF METASTASES

Table 1 shows a series of experiments that demonstrate the phenomenon, to which I have already referred, of powerful concomitant immunity to iv challenge (10). The fact that rats bearing a primary, chemically induced sarcoma are able to reject autologous tumor cells injected iv in an immunologically specific way suggests that immunologic factors may have a function in preventing the spread of

tumors by blood-borne cells. Since tumors are continually releasing viable cells into the circulation, there must be mechanisms that prevent such cells from giving rise to metastatic growths in the lung. That immune factors have a part in restraining blood-borne cells from giving rise to metastasis can be demonstrated in a number of ways. I must stress that immunity can be, at best, only one factor and may not even be the most important mechanism that determines whether a circulating cell gives rise to a metastatic lesion.

TABLE 1.—*Concomitant resistance of rats with chemically induced primary sarcomata to autologous tumor cells injected iv**

Expt No.	Animals bearing autochthonous tumor	Number of pulmonary metastases in	
		Syngeneic rats (tumor-free)	
		1	2
H2	0	0	0
H3	0	100	100
H4	1	100	100
H5	0	3	20
H6	1	43	100
H7	14	0	—
H8	200	500	500
H9	3	30	50
H10	0	0	0
H11	0	0	0
H12	20	200	200

* Injection of 5×10^5 tumor cells derived from the primary sarcoma by biopsy. In parallel experiments in which autologous tumor cells were injected im into rats with the primary tumor, there was no evidence for concomitant immunity, and injected cells invariably gave rise to a tumor at the injection site.

Several investigators have reported that when animals are deliberately immunosuppressed, metastatic spread of transplanted tumors is enhanced; text-figure 1 presents experiments from our laboratories to illustrate this phenomenon (13). Sarcomas were transplanted into the legs of rats and surgically removed 2 weeks later. In normal animals, surgery gave a 90% cure rate. However, when the tumors were implanted into animals that had been immunosuppressed, then the cure rate dropped dramatically because the tumor disseminated prior to surgery. To determine whether cell-mediated or humoral factors contributed to the control of blood-borne metastatic spread, Proctor et al. (14) performed the experiment illustrated in table 2.

Tumors were implanted into suitable rats; 2 weeks later the thoracic duct of the tumor-bearing rat was cannulated, and the thoracic duct lymph was removed continually over a period of 6 days. This maneuver ensured that the animals were deprived of both circulating immune lymphocytes and of serum macromolecules, including antibodies. Table 2 shows that this procedure caused the tumors to metastasize. Metastases could not be prevented by return to the animals of all the lymphocytes that had been drained (collateral experiments showed quite clearly that the lymphocytes being returned entered the circulating pool). On the other hand, the return of sera from tumor-bearing rats during the course of thoracic duct cannulation reversed the metastasis-inducing effect of thoracic duct drainage (for technical reasons, thoracic duct lymph

could not be returned; because there is an equilibrium between lymph, plasma, and blood, the plasma serum was returned).

We concluded from these experiments that the effector arm responsible for controlling blood-borne spread in this particular experimental situation was not circulating immune lymphocytes but rather an immunologically specific factor present in the sera of tumor-bearing rats. This deduction was confirmed by other experiments (table 3) that demonstrated it was possible to protect normal, i.e., unimmunized, rats against an iv challenge with sarcoma cells by prior injection of serum from rats with a growing tumor of the same type (14). The sera of tumor-bearing rats, however, were unable to protect against an intramuscular injection of tumor cells. The nature of the factor present in both blood and lymph plasma of tumor bears, which protects against iv challenge of tumor cells in an immunologically specific way, remains to be established. In the sera of these tumor-bearing rats, no antibody activity of the IgG or IgM class directed against the TSTA of the tumor cells could be detected, because in rats with large tumors the antibody against the TSTA was neutralized by circulating antigen (15). In the sera of rats with large tumors, antibody-antigen complexes were detected. It is, therefore, possible that antigen-antibody complexes of certain types may be able to protect against iv but not against im, challenge. In other words, far from aiding escape, antigen-antibody complexes may exert a useful antitumor action under certain conditions.

It is known (16) that antigen-antibody complexes can bind to certain types of mononuclear cells (probably monocytes) via the Fc receptor and that the cells then acquire the capacity to lyse cells bearing the antigen corresponding to the antigen-antibody complex. In other words, antibody may be used twice. First, it combines with tumor-specific antigen either in the circulation or on the surface of tumor cells. It is then released as an antigen-antibody complex, which "arms" normal circulating mononuclear cells and renders them specifically cytotoxic. I must stress, however, that regarding the protection of animals against iv-injected tumor cells by sera from tumor-bearing animals, no one has yet demonstrated that the active principle is indeed an antigen-antibody complex; perhaps it is another immunologically specific macromolecule.

WHY TUMORS VARY IN THEIR TENDENCY TO METASTASIZE SPONTANEOUSLY

The experiments just described were done with tumors that, in general, did not metastasize spontaneously and that could be cured surgically as long as the animal was not immunosuppressed. Other experimental tumors, however, give rise to spontaneous metastases, and animals bearing such tumors in the leg cannot be cured by surgical removal. Evidence has been accumulating that immune parameters have a part in determining the rate of spontaneous resistance to metastatic spread in the nonimmune suppressed host. In experimental animals, a correlation between a tendency to metastasize and immunogenicity has been observed for a series of tumors (13). Immunogenicity is measured in these experiments by the capacity of the immunized host to reject a subsequent tumor challenge. The larger the number of tumor cells that the immune host can reject, the more immunogenic the tumor is said to be. Immunogenicity is a complex parameter and is determined by at least two factors: 1) the magnitude of

TEXT-FIGURE 1.—Effect of immunosuppression on the incidence of metastases of a rat sarcoma transplanted im into the hind limb of a syngeneic recipient. Two weeks after tumor implantation, initial tumor was surgically removed. Animals were observed up to 30 weeks for the development of metastases.

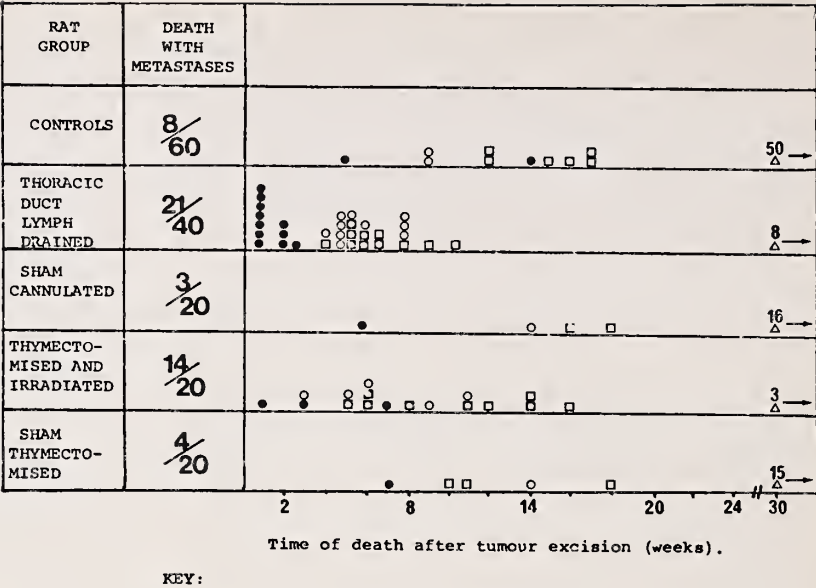


TABLE 2.—Effect of continuous removal of thoracic duct lymph from rats with an HSH sarcoma in the leg on the development of lung metastases ^a

Treatment	Series 1: Thoracic duct drained in rats with 11-day tumor		Series 2: Thoracic duct drained in rats with 18-day tumor	
	Number of rats with lung tumors	Average weight of lung tumors (g)	Number of rats with lung tumors	Average weight of lung tumors (g)
None	0/4	—	0/5	—
Thoracic duct drained	4/4	4.6	5/5	3.0
Thoracic duct drained; lymphocytes returned ^b	5/5	1.8	4/4	2.7
Thoracic duct drained; sera from rats with HSH tumor given ^c	0/4	—	1/4	0.1
Thoracic duct drained; sera from normal rats given ^c	4/4	2.4	4/4	3.4

^a Tumor was implanted in leg on day 1; thoracic duct drained from day 11-16 (series 1) or day 18-24 (series 2). Tumor was amputated immediately after draining of thoracic duct was completed, and animal was killed on day 61 (series 1) or day 66 (series 2).
^b Washed thoracic duct lymphocytes (5×10⁶) from tumor-bearing rats were injected iv daily during the period of thoracic duct draining.
^c Sera (4 ml) from rats bearing an 8-day HSH tumor (or from normal rats) were injected ip every 12 hours during the period of thoracic duct draining.

the host reaction (i.e., the amount of antibody and the number of cytotoxic cells produced by the host in response to the immunization) and 2) the effectiveness with which the tumor cells can escape from the host's immune defenses. We have compared the magnitude of the host response (i.e., antibody levels and cytotoxicity of cells in the draining nodes) with the TSTA of chemically induced tumors, which vary in their capacity to give rise to metastases, and found to our surprise that this did not vary greatly from one tumor to the next in spite of great variations in metastatic spread (17). However, the metastatic tumors are characterized by cells that readily shed their TSTA spontaneously. We measured the spontaneous shedding by culturing the cells and measuring the concentration of antigens in the

culture fluid. From these experiments we conclude that for these tumors the immune parameter that facilitates metastasis is the efficiency of escape and not the magnitude of the host reaction that is being evoked. The metastasizing tumors resemble the virulent *Pneumococci* in that both readily shed antigens spontaneously. Circulating TSTA is found in the sera of animals with nonmetastasizing tumors because there are three mechanisms by which tumor-specific, membrane-bound antigens can gain access to the circulation in a soluble form (15): 1) spontaneous release; 2) the result of autolysis of the tumor cells; and 3) the by-product of an immune attack against the tumor. A high rate of spontaneous shedding of antigens facilitates the escape of disseminated tumor

TABLE 3.—Effect of sera and lymph from tumor-bearing rats on growth in the lung of iv-injected MC-I rat sarcoma cells

Pretreatment ^a	10 ⁶ MC-I cells injected iv ^b		2×10 ⁴ MC-I cells injected im	
	Number of rats with lung tumor	Average No. of tumor nodules in lung per rat ^c	Number of animals with tumors	Average weight of tumor (g)
None	10/10	12.1 (2-31)	—	—
Sera from rats with MC-I sarcoma	5/10	2.6 (0-8)	5/5	5.8
Sera from normal rats	9/10	10.1 (0-21)	4/5	7.8
Sera from rats with antigenically unrelated MC-III sarcoma	5/5	8.6 (2-25)	—	—
Lymph plasma from normal rats	10/10	7.9 (1-14)	—	—
Lymph plasma from rats with MC-I sarcoma	6/10	2.7 (0-10)	—	—

^aSera (6 ml) were injected ip 4 hours before inoculation of tumor cells. Thoracic duct lymph (150 ml) free of cells was injected ip in five aliquots of 30 ml over a period of 24 hours 1 day before challenge with tumor cells. Sera and lymph were collected from rats that had been inoculated with a mechanically dispersed suspension of tumor 8 days previously, all of which had a growing tumor of 0.5-1 cm in diameter.

^bPrepared by trypsin digestion of a solid tumor. Rats were killed 26 days later, and tumors were weighed.

^cRange of values are in parentheses.

cells and allows them to establish themselves as metastatic growth. I must emphasize, however, that the efficiency of escape from the immune defenses of the host can, at best, be only one of several factors that determine whether cells that are continually released from the primary tumor into the circulation give rise to metastases.

CONCLUSION

In considering a phenomenon such as spontaneous regression, one should focus attention not only on changes in the activity of the host against the tumor but also on changes in the effectiveness with which the tumor cells escape. Unfortunately, escape mechanisms are complex, and our understanding of the mechanisms involved is as yet rudimentary.

REFERENCES

- (1) ALEXANDER P: Factors contributing to the "success" of antigenic tumors. In *Morphological and Functional Aspects of Immunity* (Lindahl-Kiessling K, Alm G, Hanna MG, eds.). New York, Plenum Press, 1970, pp 567-574
- (2) HELLSTRÖM I, HELLSTRÖM KE: Studies on cellular immunity and its serum-mediated inhibition in Moloney virus-induced mouse sarcomas. *Int J Cancer* 4:587-600, 1969
- (3) SjöGREN HO, HELLSTRÖM I, BANSAL SC, et al: Suggestive evidence that the "blocking antibodies" of tumor-bearing individuals may be antigen-antibody complexes. *Proc Natl Acad Sci USA* 68:1372-1375, 1971
- (4) CURRIE GA, BASHAM C: Serum-mediated inhibition of the immunological reactions of the patient to his own tumour: A possible role for circulating antigen. *Br J Cancer* 26:427-438, 1972
- (5) THOMSON DM, STEELE K, ALEXANDER P: The presence of tumour-specific membrane antigen in the serum of rats with chemically-induced sarcomata. *Br J Cancer* 27:27-34, 1973
- (6) THOMSON DM, SELLENS V, ECCLES S, et al: Radioimmunoassay of tumour-specific transplantation antigen of a chemically induced rat sarcoma: Circulating soluble tumour antigen in tumour bearers. *Br J Cancer* 28:377-388, 1973
- (7) LINNA TJ, HU C, THOMPSON KD: Development of systemic and local tumors induced by avian reticuloendotheliosis virus after thymectomy or bursectomy. *J Natl Cancer Inst* 53:847-854, 1974
- (8) CURRIE GA, GAGE JO: Influence of tumour growth in the evolution of cytotoxic lymphoid cells in rats bearing a spontaneously metastasizing syngeneic fibrosarcoma. *Br J Cancer* 28:136-146, 1973
- (9) ALEXANDER P, BENSTED J, DELORME EJ, et al: The cellular immune response to primary sarcomata in rats. II. Abnormal responses of nodes draining the tumour. *Proc R Soc Med* 174:237-251, 1969
- (10) ALEXANDER P, HALL JG: The role of immunoblasts in host resistance and immunotherapy of primary sarcomata. *Adv Cancer Res* 13:1-37, 1970
- (11) THOMSON DM, ALEXANDER P: A cross-reacting embryonic antigen in the membrane of rat sarcoma cells which is immunogenic in the syngeneic host. *Br J Cancer* 27:35-47, 1973
- (12) ALEXANDER P: A cross-reacting fetal antigen in primary chemically induced sarcomas of rats and its relation to immunotherapy. In *Conf-710527, Proceedings of the First Conference and Workshops on Embryonic and Fetal Antigens in Cancer*, Oak Ridge, Tenn., May 24-26, 1971
- (13) ECCLES SA, ALEXANDER P: The macrophage content of tumours in relation to the immune reaction of the host and metastatic spread. *Nature* 250:667-669, 1974
- (14) PROCTOR JW, RUDENSTAM CM, ALEXANDER P: A factor preventing the development of lung metastases in rats with sarcomas. *Nature* 242:29-31, 1973
- (15) THOMSON DM, ECCLES S, ALEXANDER P: Antibodies and soluble tumour-specific antigens in blood and lymph of rats with chemically induced sarcomata. *Br J Cancer* 28:6-15, 1973
- (16) PERLMANN P, PERLMANN H, MÜLLER-EBERHARD HJ: Lymphocyte-mediated cytotoxicity by humoral antibodies—mechanisms of induction and surface markers of the effector cells. *Int Arch Allergy Appl Immunol* 45:278, 1973
- (17) CURRIE GA, ALEXANDER P: Spontaneous shedding of tumour-specific antigens by viable sarcoma cells: Its possible role in facilitating metastatic spread. *Br J Cancer* 29:72-75, 1974

Spontaneous Tumor Regression: Possible Relationship to In Vitro Parameters of Tumor Immunity^{1,2}

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SUMMARY—Evidence is discussed from three animal systems (Shope papillomas in rabbits, Moloney sarcomas in mice, Schmidt-Ruppin-Rous sarcomas in Japanese quail) that immune reactions can be important in spontaneous tumor regression. In vitro studies performed in these systems indicate that blocking serum factors can thwart cell-mediated immune responses and that “unblocking” antibodies are often found in animals whose tumors have regressed. To what extent spontaneous regressions of human neoplasms are due to immunologic mechanisms is unknown; in 2 patients who had undergone spontaneous tumor regression, tumor cell cytotoxic lymphocytes and unblocking serum factors were detected. Better animal models and better knowledge about the mechanisms of tumor immune reactions are needed before tumor regression can be more successfully induced by immunologic manipulations (to what extent that will even be feasible in man is unknown).—*Natl Cancer Inst Monogr* 44: 131–134, 1976.

The fact that spontaneous regression of human cancer occasionally occurs has raised hopes that if the mechanisms of this regression were known, tumor regression could be induced at will. Cell biologists may speculate that the mechanisms of regression involve changes in some growth-controlling cellular mechanism, and endocrinologists may ascribe it to some hypothetical hormone changes. Immunologists will, instead, argue that host defense mechanisms against tumor-associated antigens are likely to be involved. The fact that spontaneous regression of human tumors is utterly rare makes it difficult to find out which of these possible explanations, if any, is correct; probably, there is a multitude of causes of spontaneous tumor regressions, including immunologic ones.

We will first review some immunologic studies on cell-mediated and humoral immune responses to tumor antigens and will then discuss some findings obtained in three animal tumor systems in which spontaneous tumor regressions occur regularly. Finally, we will mention some similar studies performed in man.

CELL-MEDIATED IMMUNITY TO TUMOR ANTIGENS

Lymphocytes have a major function in the body's immunologic defense to antigenic tumors (1–3). This is apparent from the fact that effective tumor immunity can be adoptively transferred with lymphoid cells and that lymphocytes from immune animals can kill the respective neoplastic cells in vitro. Since this work has been repeatedly reviewed (1–3), we will not present the evidence for cell-mediated tumor immunity here.

In most cases, lymphocytes from tumor-bearing individuals are cytotoxic to cells from the respective tumors in vitro, whereas in a few instances, either no cytotoxic effect or only a weak one is seen with tumor-bearer lymphocytes.

Sera from tumor-bearing individuals commonly possess “blocking factors” capable of thwarting the lymphocyte-mediated immune response (1–3). These blocking factors have been found to exert their action on the effector cells primarily, although they can also bind to the tumor cells and be absorbed from serum with them. In many cases, the blocking factors appear to be complexes between tumor antigens and IgG antibodies to these antigens (3, 4); in other cases, antigen alone has been found capable of blocking at the effector cell level (3, 4–6).

Antibodies to tumor antigens can occasionally mask the antigens of the neoplastic cells and so protect these from attack by sensitized lymphocytes (3, 7). They may also aid in the detachment of tumor antigens, blocking at the effector cell level, either alone or after having been complexed with antibodies (8, 9). In many cases, however, no blocking effect of free antibodies has been detected (3). Rather, such antibodies have been found capable of specifically cancelling the blocking activity of tumor-bearer serum, an effect often referred to as “unblocking.” The unblocking antibodies can be removed by absorption with the respective tumor cells (3). These antibodies probably act by combining with the antigenic part of the blocking factors and by preventing their interaction with the effector cells (3).

Pollack et al. (10) and Skurzak et al. (11) found that certain sera from tumor-immune donors contain lymphocyte-dependent antibodies, which can bestow a cytotoxic effect on lymphocytes from nonsensitized hosts. This phenomenon is similar to that described by Perlmann and Holm (1) and by McLennan et al. (12), who studied immunity to alloantigens and xenoantigens.

The cells acting as effectors in antibody-dependent, lymphocyte-mediated cytotoxic reactions appear to be primarily “K” (killer) cells, lacking the theta marker as well as cell-surface immunoglobulin markers and having receptors for antigen-antibody complexes (3). In some cases, monocytes may also act as effectors in these reactions. In vivo, lymphocytes carrying the theta marker appear to be important, at least in the defense against certain tumors. In vitro, killing of tumor cells by both theta bearing lymphocytes and K cells has been observed. The tumor systems studied, the techniques used, and the time point of lymphocyte harvest seem to have greatly influenced whether primarily T or K cell killing was observed (3).

In vivo, the situation appears to be quite complex. In addition to direct killing of tumor cells by sensitized lymphocytes, indirect mechanisms involving the production of various lymphokines and the participation of macrophages are probably involved (3). Nonspecifically acting blocking factors, including alpha globulins and toxic products released from tumor cells, may be involved in addition to the specific blocking factors (3). Furthermore,

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anatomic barriers between tumors and immunologically competent cells may aid in the escape of the tumors from immunologic control.

There is some evidence that both specific and non-specific stimulation of the immune response to tumor antigens can have an immunotherapeutic effect (3). In some animal systems, this evidence is very strong. On the other hand, an unequivocal demonstration of immunotherapeutic success in man remains to be seen. In virtually all immunotherapeutic trials, in animals as well as in man, no detectable response has been obtained when the tumor load has been large. This might be because the local environment in the area of a large tumor contains such high amounts of blocking factors, including free antigens and antigen-antibody complexes, that lymphocytes entering it will be automatically deflected from reacting.

With this background, we will now discuss some immunologic studies performed with three types of animal neoplasms that undergo spontaneous regression in a predictable fashion.

SPONTANEOUS REGRESSION OF ANIMAL NEOPLASMS

We present three tumor systems that we have been studying ourselves: Shope papillomas in rabbits, Moloney virus-induced sarcomas in mice, and Schmidt-Ruppin-Rous virus-induced sarcomas in Japanese quail.

The Shope virus induces papillomas, some of which persist for a long time and then progress to carcinomas. Other papillomas regress, after which the rabbits are resistant to further Shope papilloma induction. The fact that DNA from the Shope virus can induce papillomas in persisters rabbits (as it can do in untreated animals), whereas it cannot induce tumors in regressors, indicates that there is a difference, between the immune status of the regressors and the persisters (13).

A lymphocyte-mediated cytotoxic effect on cultivated Shope tumor cells was detected when studying both regressors and persisters. Titrations of the lymphocyte effect have not been done, however, so quantitative differences in reactivity between the two groups of animals cannot be ruled out. Sera from the persisters, but not from the regressors (or from untreated rabbits), were found to block lymphocyte-mediated killing of Shope tumor cells (14). This indicated that the mechanism of spontaneous regression of the Shope papillomas may be of an immunologic nature. It does not prove this interpretation to be correct, however. The fact that "vaccination" of rabbits with Shope tumor extracts increases the regression frequency (15) and that treatment with immunosuppressive drugs decreases it (16) provides more direct evidence for an immunologic mechanism being responsible for tumor regression in this system.

Data obtained with Moloney sarcomas in mice (17) are similar, in that cytotoxic lymphocytes and blocking serum factors can be detected with the microcytotoxicity assay, the blocking factors being primarily seen in sera from mice with growing Moloney sarcomas ("progressors"). Sera from mice with tumors that have started to regress contain antibodies cytotoxic to Moloney sarcoma cells in the presence of complement (18-20). Such sera are often also unblocking (21), i.e., they can cancel the blocking effect of sera from mice with growing Moloney sarcomas.

Inoculation of lymph node and spleen cells from regressors, or of regressor serum, has led to tumor regression in about one-third of the mice receiving Moloney sarcoma virus at an early age (less than 3 weeks) when they would

have been normally unable to reject the tumors (18). The individual roles of cytotoxic antibodies and complement, of specifically sensitized lymphocytes, of various inflammatory cells, and of unblocking versus lymphocyte-dependent antibodies in the tumor regression remain unknown. Nevertheless, the data establish that immune mechanisms, probably involving both sensitized cells and antitumor antibodies, are responsible for the spontaneous regression of Moloney sarcomas.

When Japanese quail are inoculated with the Schmidt-Ruppin variant of Rous sarcoma virus, some animals die from progressively growing tumors, whereas others recover with their tumors undergoing spontaneous regression. Lymphocyte-mediated tumor cell cytotoxicity has been detected only in those animals with regressed sarcomas, but we have not been able, despite various attempts to manipulate the effector cells *in vitro*, to detect any cell-mediated immunity in the progressors (22).

Sera from progressor quail can, in most cases, block lymphocyte-mediated killing of the Rous sarcoma cells. Some regressor sera are also blocking (23), but their blocking titer is generally lower than that of progressor sera. Furthermore, there is evidence that the mechanisms of the blocking activity of progressor and regressor sera differ: Progressor sera can block tumor cell killing by interacting with the effector cells, while most (albeit not all) of the blocking effect of regressor sera appears to involve interaction between the sera and the tumor cells, probably releasing antigens which then (alone or as antigen-antibody complexes) act on the effector cells (9).

Thymectomy of Japanese quail prevents spontaneous regression of Rous sarcomas (24). This finding, together with the *in vitro* data, supports the notion that the mechanisms of spontaneous tumor regression is immunologic also in this system.

Are there human counterparts of the three animal systems now discussed? The fact that spontaneous tumor regression in man is utterly rare makes it difficult to answer this question. We have studied only two patients who have undergone spontaneous tumor remission.

One patient, M.L., had a colon adenocarcinoma, which had metastasized to the peritoneal cavity and to the liver. Following a low-dose therapy with 5-fluorouracil (which was not expected to be curative), the metastatic tumor masses disappeared. This patient is still living, 9 years later. Repeated tests on M.L. have demonstrated a cytotoxic effect of his blood lymphocytes on cultivated colon carcinoma cells and an unblocking effect of his serum, when tested in combination with serum from patients with growing colon carcinomas (25).

Another patient, M.D., had a malignant melanoma with lymph node metastases, which regressed 5 years ago without any therapy being given. Blood lymphocytes from this patient are cytotoxic to melanoma cells *in vitro*, and M.D.'s serum is unblocking (26). Furthermore, antibodies binding to the surface of melanoma cells have been consistently demonstrated in M.D.'s serum. These antibodies have not reacted with various control tumor cells tested, whereas they have reacted with all of eight melanoma target cell lines assayed.

One may speculate that, to the extent tested, there is a similarity between those animal tumor systems in which spontaneous tumor regression was studied and the patients similarly investigated. It is not possible to conclude, however, that the unblocking antibodies detected were, indeed, responsible for tumor regression and were not just epiphe-

nomena, since if, for example, regression had resulted from a nonimmunologic event, removal of the source of tumor antigen would still be expected to decrease serum blocking activity and perhaps turn the serum to an unblocking one. The situation would become more clear if one could transfer effective tumor immunity with cells, transfer factor, or serum from the patients whose tumors regressed. Obviously, many logistic problems make studies of that type difficult. If effective immunity could be transferred, this would be fairly conclusive, but if it could not, it may well have been so that the "right" type of immunity was not transferred, the doses of serum cells or transfer factor were too low, etc.

OUTLOOK

Better knowledge of the mechanisms of the immune response to tumor antigens, of the cells and molecules involved, and of their mode of action, will hopefully provide better opportunities for changing this response in such a way that it becomes more effective *in vivo*. Appropriately selected animal models, including those which have cross-reacting tissue type-specific tumor antigens, may be helpful in these studies, including, for example, chemically induced urinary bladder papillomas and carcinomas of rats and chemically induced colon carcinomas of rats (3). Using such models, one may investigate whether, indeed, one could induce tumor regression at will. The fact that the amount of success has been small so far does not necessarily have to mean that the problem is insurmountable (and it is at least a better working attitude to believe that it is not).

One approach which our laboratory has taken is to study immune reactions to embryonic tumor antigens, concentrating on those antigens that can be detected by the sensitivity of tumor cells *in vitro* to exposure to lymphocytes from pregnant, syngeneic animals (3). Our reason for choosing this system is that there is, on the one hand, evidence that an immune response can be detected *in vitro* while, on the other hand, this response appears to be very ineffective in counteracting tumor growth *in vivo*. As we have published elsewhere (27, 28), sera from multiparous syngeneic mice, from tumor-bearing mice, and from mice immunized to the tumor-specific antigens of chemically induced sarcomas (and showing transplantation resistance against these) can block destruction of cultivated neoplastic cells by lymphocytes from multiparous mice.

One provocative observation made during these studies is that embryonic tumor antigens appear to be more capable than are individually unique tumor-specific transplantation antigens in eliciting a strong blocking reaction. The possibility should, therefore, be considered that differences in the ability of different antigens to block cell-mediated immune responses (alone, or as part of antigen-antibody complexes) may constitute one of the most important distinctions between those antigens which are good transplantation antigens and those which are not. If this assumption is true, one should exert much effort to increase effective tumor immunity by attempting to circumvent the blocking mechanism, e.g., by giving unblocking sera or inoculating lymphocytes which have a level of sensitization such that this cytotoxic activity is relatively resistant to the blocking effect (3). Experiments are in progress to study this; whether they will be successful is obviously unknown.

REFERENCES

- (1) PERLMANN P, HOLM G: Cytotoxic effects of lymphoid cells *in vitro*. *Adv Immunol* 11:117-193, 1969
- (2) BALDWIN RW: Immunological aspects of chemical carcinogenesis. *Adv Cancer Res* 18:175, 1973
- (3) HELLSTRÖM KE, HELLSTRÖM I: Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. *Adv Immunol* 18:208-277, 1974
- (4) SJÖGREN HO, HELLSTRÖM I, BANSAL SC, et al: Suggestive evidence that the "blocking antibodies" of tumor-bearing individuals may be antigen-antibody complexes. *Proc Natl Acad Sci USA* 68:1372-1375, 1971
- (5) CURRIE GA, BASHAM C: Serum mediated inhibition of the immunological reactions of the patient to his own tumour, a possible role for circulating antigen. *Br J Cancer* 26:427-438, 1972
- (6) BALDWIN RW, PRICE MR, ROBINS RA: Inhibition of hepatoma-immune lymph-node cell cytotoxicity by tumour-bearer serum and solubilized hepatoma antigen. *Int J Cancer* 11:527-535, 1973
- (7) ROBINS RA, BALDWIN RW: Tumour-specific antibody neutralization of factors in rat hepatoma-bearer serum which abrogate lymph-node-cell cytotoxicity. *Int J Cancer* 14:589-597, 1974
- (8) THOMSON DM, STEELE K, ALEXANDER P: The presence of tumour-specific membrane antigen in the serum of rats with chemically induced sarcomata. *Br J Cancer* 27:27-34, 1973
- (9) HAYAMI M, HELLSTRÖM I, HELLSTRÖM KE, et al: Further studies on the ability of regressor sera to block cell-mediated destruction of Rous sarcomas. *Int J Cancer* 13:43-53, 1974
- (10) POLLACK S, HEPPNER G, BRAWN RJ, et al: Specific killing of tumour cells *in vitro* in the presence of normal lymphoid cells and sera from host immune to the tumour antigens. *Int J Cancer* 9:316-323, 1972
- (11) SKURZAK HM, KLEIN E, YOSHIDA TO, et al: Synergistic and antagonistic effect of different antibody concentrations on *in vitro* lymphocyte cytotoxicity in the Moloney sarcoma virus system. *J Exp Med* 135:997-1000, 1972
- (12) MACLENNAN JC, LOEWI G, HARDING DB: The role of immunoglobulins in lymphocyte-mediated cell damage *in vitro*. I. Comparison of the effects of target cell specific antibody and normal serum factors on cellular damage by immune and non-immune lymphocytes. *Immunology* 18:397-404, 1970
- (13) EVANS CA, ITO Y: Antitumor immunity in the Shope papilloma-carcinoma complex of rabbits. III. Response to reinfection with viral nucleic acid. *J Natl Cancer Inst* 36:1161-1166, 1966
- (14) HELLSTRÖM I, EVANS CA, HELLSTRÖM KE: Cellular immunity and its serum mediated inhibition in Shope virus induced rabbit papillomas. *Int J Cancer* 4:601-607, 1969
- (15) EVANS CA, GORMAN LR, ITO Y, et al: Antitumor immunity in the Shope papilloma-carcinoma complex of rabbits. I. Papilloma regression induced by homologous and autologous tissue vaccines. *J Natl Cancer Inst* 29:277-285, 1962
- (16) ALLISON CA, FRIEDMAN RM: Effects of immunosuppressants on Shope rabbit fibroma. *J Natl Cancer Inst* 36:859-868, 1966
- (17) HELLSTRÖM I, HELLSTRÖM KE: Studies on cellular immunity and its serum-mediated inhibition in Moloney virus-induced mouse sarcomas. *Int J Cancer* 4:587-600, 1969
- (18) HELLSTRÖM I, HELLSTRÖM KE, PIERCE GE, et al: Studies on immunity to autochthonous mouse tumors. *Transplant Proc* 1:90-94, 1969
- (19) LAMON EW, KLEIN E, ANDERSSON B, et al: The humoral antibody response to a primary viral neoplasm (MSV) through its entire course in BALB/c mice. *Int J Cancer* 12:637-645, 1973
- (20) TAMERUS J, HELLSTRÖM I: *In vitro* demonstration of cytotoxic antibodies to Moloney sarcoma cells. *J Immunol* 112:1987-1996, 1974
- (21) HELLSTRÖM I, HELLSTRÖM KE: Colony inhibition studies on blocking and non-blocking serum effects on cellular immunity to Moloney sarcomas. *Int J Cancer* 5:195-201, 1970
- (22) HAYAMI M, HELLSTRÖM I, HELLSTRÖM KE, et al: Cell-mediated destruction of Rous sarcomas in Japanese quails. *Int J Cancer* 10:507-517, 1972
- (23) HAYAMI M, HELLSTRÖM I, HELLSTRÖM KE: Serum effects on cell-mediated destruction of Rous sarcomas. *Int J Cancer* 12:667-688, 1973
- (24) YAMANOUCHI K, HAYAMI M, MIYAKURA S, et al: Cellular immunity induced by Rous sarcoma virus in Japanese quail. II. Effect of thymectomy and bursectomy on oncogenesis of Rous sarcoma virus. *Jpn J Med Sci Biol* 24:1-8, 1971

- (25) HELLSTRÖM I, HELLSTRÖM KE, PIERCE GE, et al: Cellular immunity to colon carcinomas in man. *In* Carcinoma of the Colon and Antecedent Epithelium (Burdette WJ, ed.). Springfield, Ill., Charles C Thomas, 1970, pp 176-188
- (26) HELLSTRÖM I, HELLSTRÖM KE, STÖGREN HÖ, et al: Serum factors in tumor-free patients cancelling the blocking of cell-mediated tumor immunity. *Int J Cancer* 8:185-191, 1971
- (27) HELLSTRÖM I, HELLSTRÖM KE: Cytotoxic effect of lymphocytes from pregnant mice on cultivated tumor cells. I. Specificity, nature of effector cells, blocking by serum. *Int J Cancer* 15:1-16, 1975
- (28) ———: Cytotoxic effect of lymphocytes from pregnant mice on cultivated tumor cells. II. Blocking and unblocking of cytotoxicity. *Int J Cancer* 15:30-38, 1975

Mechanisms of Escape From Immune Surveillance^{1,2}

George Klein³

SUMMARY—The escape from immune rejection is, like the development of drug resistance in microorganisms, a multi-pathway process. Selection favors the phenotype, i.e., the cell that can grow in spite of the immune rejection process, not the underlying mechanism. Experimental evidence documents the roles of host immunosuppression (genetic or environmental), immunoresistance at the cellular level, “sneaking through” (i.e., growth of a tumor to irreversible size prior to the mobilization of an appropriate immune response), lack of antigenic recognition, and blocking enhancement type reactions.—*Natl Cancer Inst Monogr* 44: 135-136, 1976.

I have a strong feeling that in this session, which has a doubly redundant title in contrast to the rather precise titles of the previous sessions, we are asked to discuss something like, say, pigment formation in the white elephant, or the white whale, or the social behavior of a community of whales when encountering a white whale. All we can provide, in terms of experimental evidence, are our own black mice, because this is the only animal that fits our cages. Having said that, however, I will continue in the same vein as the other speakers.

Many translators to the Hungarian language often try their skill on one great classic poem (like “The Raven” by E. A. Poe). They generally come up with very different results. In the same way, each tumor immunologist has his own list of escapes from rejection. Mine is similar to Peter Alexander’s (1). There are a few additional things that I would like to emphasize. There is the curious phenomenon of “sneaking through,” which may be related to some of the phenomena that Dr. Pierce discussed (2). It also illustrates the importance of the critical tumor size and the invulnerability of the tumor to immune attack after it has reached a certain size, perhaps after it has been vascularized. The term itself was coined by Old et al. (3) to describe the situations in which small antigenic inocula grow progressively, medium-sized inocula are rejected, and large inocula break through again. We need to learn a great deal about the mechanism of this phenomenon. It is possible that it is a simple numbers game, whereby tumor growth outpaces the mobilization of the rejection response. On the other hand, it is conceivable that more specific phenomena, e.g., low zone tolerance, may be involved as well (4).

Another mechanism that has not been mentioned in great detail is *immunoresistance*. I am quite convinced about its reality. If a population of tumor cells is forced to grow in the presence of prolonged immune pressure, we invariably

end up with a population of variants that are less rejectable under the same circumstances. As a rule, they have a decreased concentration of the target antigen. Our recent analysis of certain somatic cell hybrids (5) showed that, whereas decreased antigen concentration leads to a relative immunoresistance, the opposite is not always true. The low antigen expression of an immunoresistant cell was recessive, i.e., high antigenicity was reestablished by hybridization with a different type of cell. Nevertheless, some of the hybrids remained immunoresistant, which showed that membrane properties, other than antigen concentration per se, may influence the sensitivity of the tumor cell to humoral and/or cell-mediated immunity.

I would also like to stress the *lack of detectable antigenicity* encountered with certain tumors. This is to remind you of the genetic recognition systems I mentioned earlier in this meeting (6). In addition to the examples of Baldwin, mentioned earlier, there is at least one virally induced tumor, murine sarcoma virus (MuSV)-induced mouse sarcoma, in which antigenic activity seems to be determined by the helper virus rather than the transforming virus itself (7, 8). It must be remembered, however, that MuSV probably never occurs in nature without a helper virus. It is possible that no surveillance has developed against the S+L— (sarcoma virus-positive, leukemia virus-negative) type of tumor, because this form can only be established in the laboratory under highly artificial circumstances. I would still like to entertain the notion that the S+L— cells have a membrane change, associated with their lack of contact inhibition and their neoplastic growth in vivo, but this is not recognized by the host as an antigen because the host has not been selected for the corresponding immune recognition. A similar reasoning may apply to many of the spontaneous or chemically induced tumors that are not detectably immunogenic. The responses that *can* be found against the relatively highly antigenic, chemically induced tumors may be comparable with the good immune responses that can be encountered in relation to certain entirely artificial haptens. In the absence of appropriate selection, it would be a matter of chance whether such a situation arises, with good recognition as the result.

Speaking about the *malfunctioning of the effector mechanisms*, I would go along with most of the previous speakers who have emphasized that antigen release from the tumor was a most important factor in paralyzing the rejection response. I would like to come back to Burkitt’s lymphoma. I think that this model is relevant, not only because occasional spontaneous regressions do occur (9) but also because of the regression of Epstein-Barr virus (EBV)-induced infectious mononucleosis. This perfectly regular event may illustrate an efficient rejection reaction operating on the basis of appropriately selected and probably multiple recognition systems. Carter (10) has emphasized the similarity between malignant lymphoma and the extensive infiltration of the inner organs with atypical lymphoid cells during acute infectious mononucleosis.

Since EBV can transform normal peripheral lymphocytes

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with a limited life-span into "immortal," potentially malignant cells in vitro (11), the question is not so much why the EBV-carrying cells grow in vivo as why they stop growing. The atypical cells in the peripheral blood are in a large part T cells (12), probably sensitized against EBV-carrying, potentially malignant cells (Svedmyr E, Jondal M: Unpublished data). It is conceivable that they are the effectors of a rejection reaction.

We have studied (13, 14) the antibody response against EBV-determined membrane antigens (MA) in a number of Burkitt's lymphoma patients whose tumors have gone to apparently total regression after chemotherapy but recurred after relatively long periods of time. In patients with maintained regression, the anti-MA level tends to maintain a fairly steady level throughout many years, like in EBV-infected normal persons, although at a higher titer. In the patients with recurrence, the membrane-reactive antibody level often drops several months prior to the clinically diagnosed recurrence. When the tumor recurs, the anti-MA level tends to rise again. There is evidence (13, 14) suggesting that the antibody drop is due to the appearance of antigen-antibody complexes, often leading to antigen excess, with EBV-determined MA specificity. Since the anti-MA level tends to rise again after the tumor has recurred, it is possible that the prerenal antigen, released from an unknown reservoir, is capable of binding antibody but is nonimmunogenic. Although it may block cell-mediated immune responses, it cannot induce an anamnestic response, in contrast to the membrane constituents released by the growing tumor. It is important to establish the source of the prerenal antigen. Preliminary evidence suggests that it is due not to viremia but to the release of cell-associated membrane antigen fragments, perhaps from latent tumor foci.

The great merit of the Burkitt system lies in the fact that a serologic definition of the appropriate antigen-antibody system is possible. A similar serologic definition of other tumor-associated antigen-antibody systems will be essential before some of the crucial events during tumor regression

and recurrence can be subjected to appropriate immunologic scrutiny.

REFERENCES

- (1) ALEXANDER P: Metastatic spread and "escape" from the immune defenses of the host. *Natl Cancer Inst Monogr* 44:125-129, 1976
- (2) PIERCE GB, FENNELL RH Jr: Latent carcinoma and carcinoma in situ. *Natl Cancer Inst Monogr* 44:99-102, 1976
- (3) OLD LJ, BOYSE EA, CLARK DA, et al: Antigenic properties of chemically induced tumors. *Ann NY Acad Sci* 101:80-106, 1962
- (4) KÖLSCH E, MENGENSEN R, DILLER E: Low dose tolerance preventing tumour immunity. *Eur J Cancer* 9:879, 1973
- (5) KLEIN G, FRIBERG S JR, WIENER F, et al: Hybrid cells derived from fusion of TA3-Ha ascites carcinoma with normal fibroblasts. I. Malignancy, karyotype, and formation of isoantigenic variants. *J Natl Cancer Inst* 50:1259-1268, 1973
- (6) KLEIN G: Immune surveillance—a powerful mechanism with limited range. *Natl Cancer Inst Monogr* 44:109-113, 1976
- (7) STEPHENSON JR, AARONSON SA: Antigen properties of murine sarcoma virus-transformed BALB/3T3 nonproducer cells. *J Exp Med* 135:503-515, 1972
- (8) STROUK V, GRUNDNER G, FENYÖ EM, et al: Lack of distinctive surface antigen on cells transformed by murine sarcoma virus. *J Exp Med* 136:344-352, 1972
- (9) BURKITT DP, KYALWAZI SK: Spontaneous remission of African lymphoma. *Br J Cancer* 21:14-16, 1967
- (10) CARTER RL: The pathology of infectious mononucleosis—a review. In *Oncogenesis and Herpesviruses* (Biggs RM, de-Thé G, Payne LN, eds.). Lyon, International Agency for Research on Cancer, 1972, pp 230-238
- (11) MILLER G: Human lymphoblastoid cell lines and Epstein-Barr virus: A review of their interrelationships and their relevance to the etiology and leukoproliferative states in man. *Yale J Biol Med* 43:358-384, 1971
- (12) DENMAN AM, PELTON BK: Control mechanisms in infectious mononucleosis. *Clin Exp Immunol* 18:13-25, 1974
- (13) GUNVÉN P, KLEIN G, CLIFFORD P, et al: Epstein-Barr virus-associated membrane-reactive antibodies during long-term survival after Burkitt's lymphoma. *Proc Natl Acad Sci USA* 71:1422-1426, 1974
- (14) MUKOJIMA T, GUNVÉN P, KLEIN G: Circulating antigen-antibody complex associated with Epstein-Barr virus in recurrent Burkitt's lymphoma. *J Natl Cancer Inst* 51:1319-1321, 1973

Possible Mechanisms in Regression¹

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SUMMARY—The mechanisms responsible for spontaneous regression of cancer may be nonimmunologic. Among other possible models, it is suggested that the necrotizing properties of endotoxin offer an open approach to the problem.—*Natl Cancer Inst Monogr* 44: 137-139, 1976.

The strong possibility, amounting almost but not quite to a likelihood, that immunologic mechanisms are causally involved in the phenomenon of spontaneous regression has already been dealt with authority and in some detail by others. There is little that I can add to the substance of the argument, but perhaps it would be useful to suggest several alternative possibilities which might be kept in reserve, just in case the main argument turns out to be wrong.

Concerning the immunologic explanation itself, I think it safe to say that if it is true that spontaneous regression of human cancer occurs, as has been convincingly reported in this symposium, the present level of our understanding of immunobiology is not yet sufficiently advanced so that we can explain it in any meaningful detail. In short, we are still too ignorant, and much more remains to be learned about fundamental immunologic mechanisms. Whether an established cancer is eliminated by the operations of cellular immunity, or by humoral antibody, with or without complement, or by a concert of all these acting together, we still lack the kind of information that would tell us why it works in the very rare instances when it does, and even more perplexing, why it doesn't work more frequently and more effectively. Indeed, if the problem is simply that these are foreign cells recognizable by the host as such, why doesn't it work all the time?

Perhaps it is true, as was suggested a good many years ago (1), that it does work much more frequently than we give it credit. It is still conceivable that many of the normal cells of a metazoan population have a tendency to transform spontaneously into malignant form under the influence of viruses or various chemical carcinogens and that we never see evidence of this because of the high efficiency of cellular immune mechanisms for recognizing the malignant cells and eliminating them as soon as they appear. This is the "surveillance mechanism" postulated some years back but still in need of experimental confirmation.

It is the failure of any such mechanism in the vast majority of patients who do develop cancer that needs a better explanation than I can think of. If this is the natural way for the body to rid itself of neoplastic cell populations, what is it that gets in the way? Does the cancer tissue protect itself with built-in mechanisms of its own, or does the failure simply mean that the host is lacking some essential piece of the mechanism? It could well be the latter, of course, in view of the remarkable incidence of neoplasia in patients with immunodeficiency diseases as well as in

patients subjected to organ transplantation who develop cancer in the course of immunosuppressive therapy. These patients are especially illuminating and have strengthened greatly the whole idea that immunology is a principal defense against cancer, since their tumors undergo spontaneous regression as soon as the immunosuppressive drugs are stopped. There is the self-evident caution in this context that we do not know whether the rejection mechanism is directed against the cancer as cancer, or is simply the rejection of a foreign tissue because it carries foreign histocompatibility antigens.

The possibility exists that cancer protects itself, and various mechanisms for this have been proposed. There is one of these that I would like to mention specifically, since we may have a model for studying it in some detail in a neoplastic system unrelated to cancer. This is the syncytial trophoblast of the mammalian placenta, a grafted tissue that manages to invade the maternal host and maintain its integrity for the full term of pregnancy without being threatened by rejection except under pathologic circumstances. There are a number of hypothetical explanations for this, ranging from local hormonal influences to special constituents at the surfaces of trophoblast cells which protect them against immunologic recognition; the status of this aspect of the problem has recently been summarized by Beer and Billingham (2).

The phenomenon which I should like to call to your attention is an old one, but perhaps ready for reassessment. Fragments of the syncytial trophoblast are continually coming loose in the huge vascular compartment of the placenta and floating off into the maternal circulation (3). We looked into the phenomenon by taking samples of venous blood from catheters placed in the maternal vena cava at the level of entry of the uterine veins, and we found that there are always enormous, multinucleated cells, indistinguishable from the syncytium as it appears in sections of the intervillous space. The cell masses vary in size, some containing as many as 40 nuclei. They are easily distinguished from megakaryocytes, which by the way also exist in vena caval blood in surprisingly great numbers. Although our methods for finding trophoblast cells on Millipore membranes were crude and in no sense quantitative, we always found at least one such cell in every 5-ml sample of vena caval blood. The cells were present at all stages of pregnancy, from the sixth week through to term. In other words, there is a constant, heavy shower of fetal trophoblast cells being injected into the mother's blood throughout pregnancy, amounting to a truly massive infusion, a virtual invasion by these cells.

It is difficult to view this as a biologic accident due, say, to faulty construction of syncytial trophoblast. There are reports in the pathologic literature of the finding of syncytial cells entrapped in the small pulmonary arteries and capillaries of women dying from various reasons during pregnancy, but the mystery is that the lungs are not in fact filled with such cells. It is likely that they disintegrate very rapidly, either in transit toward the lung or soon after

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landing there. In this regard, the syncytial trophoblast is rapidly lysed on exposure to proteolytic enzymes, and perhaps this is a mechanism for their dissolution in vivo (3).

The issue in the present context is that this continual shedding of cell masses from the trophoblast may be a mechanism for continual desensitization of the maternal host against any antigens possessed by this fetal tissue. If so, a parallel mechanism may exist for the shedding of cancer cells into the blood from neoplastic tissue, and the underlying purpose of the shedding process may be the same in both situations. In any case, it might be worth taking a new look at the phenomenon in pregnancy.

The frequently reported association between infections of various sorts and spontaneous regression of cancer has raised for a great many years—since before the turn of the century—the possibility that gram-negative bacterial endotoxin has a function in the phenomenon. This remains the presumed basis for the action of Coley's toxins, and the capacity of endotoxin to cause hemorrhagic necrosis in various tumors of experimental animals is now a familiar laboratory observation.

For various reasons, some of them alluded to elsewhere in this symposium, the effects of endotoxin on cancer tissue in vivo are widely regarded as having some sort of immunologic basis. This may be true, but only to a limited and perhaps even marginal extent. In my opinion, the properties of endotoxin that lead to hemorrhage, necrosis, and shock are more likely quite independent of all known immunologic mechanisms.

Time does not permit my going into all my reasons for this prejudice, and I shall therefore settle on the reactions caused by endotoxin in the horseshoe crab (*Limulus polyphemus*), an animal that, although unendowed with lymphocytes, antibody, complement, or any other recognizable immunologic equipment, is among the most sensitive of all creatures to the lethal effects of endotoxin, as was beautifully demonstrated by Bang (4) some years ago.

A parenteral injection of a small dose of endotoxin, either in the form of semipurified lipopolysaccharide or Westphal's more refined lipid A component, causes a lethal reaction in the horseshoe crab, which is remarkable for its resemblance, in certain essential features, to that in the rabbit. The vascular compartments become blocked by great aggregates of agglutinated hemocytes, which are enmeshed together by a solid gel. In essence, this is what happens to the rabbit in the course of the generalized Shwartzman reaction (5). The circulation comes to a standstill, and the *Limulus* dies within a few hours after the injection. The central event in the killing appears to be the coagulation of a *Limulus* protein, which is normally contained inside granules in the animal's hemocytes.

The clotting mechanism seems also to be involved in the necrotizing effects of endotoxin in rabbits, in the genesis of the Shwartzman reaction, and in the tumor-necrotizing property of endotoxin in mice with neoplasia. How it works is not clear, but we do know that endotoxin causes hypercoagulability of whole blood, activates Hageman factor, does something at the surfaces of platelets which results in their becoming sticky and clumped together, and somehow also causes leukocytes to become stuck to each other and to the surfaces of endothelial cells. All these events happen, and as far as the cancer is concerned, they combine to demonstrate that the best and simplest of all ways to bring about the selective destruction of neoplasia in a living animal is to bring its internal blood supply to a standstill. However, the nature of the reaction remains a

mystery. Perhaps one of our major difficulties lies in the very complexity of the coagulation mechanism as it operates in vertebrates, making it difficult to sort out among the numerous reactions the one (or ones) that is primarily affected by endotoxin.

For this reason, it may be worth having a closer look at the *Limulus* gelation reaction, on the off chance of seeing something in this presumably simpler system that may be applicable to the more complex events in tumor-bearing mammals. There are several reasons to suspect that the clottable protein in *Limulus* lysate may represent a primitive analogue of more modern coagulation systems.

1) The clotting by endotoxin is a dose- and time-dependent reaction which involves at least two sequential stages. The first stage, revealed by Levin and Bang (6), consists in the activation of a thermolabile protein, regarded by them as an enzyme. This stage is set off by the presence of extremely small concentrations of endotoxin; as little as 0.5 ng of purified lipopolysaccharide/ml is adequate, and tenfold less of Westphal's lipid A, devoid of polysaccharide side chains, is even more active. The reaction of clotting occurs in as short a time as 20 minutes with high concentrations, and takes 2 hours or longer with the smallest doses. Divalent ions are necessary for clotting; EDTA will prevent clotting by endotoxin.

2) Thromboplastin does not cause clotting of *Limulus* lysate, but it does produce a heavy flocculent precipitate within a few minutes at 37° F (Thomas L, McGregor D: Unpublished observations). This kind of reaction can be demonstrated with rabbit or mouse brain thromboplastin, devoid of endotoxin; it is not coagulation but a heavy precipitate.

3) The endotoxin-*Limulus* clotting reaction is completely prevented by small concentrations of heparin (Thomas L, McGregor D: Unpublished observations). We first observed this 2 years ago but only in a fragmentary way that was nearly impossible to reproduce at will. Also, when first seen, it was alarmingly paradoxical, in the sense that heparin would prevent coagulation by high concentrations of endotoxin but seemed to have no protective effect when added in the presence of very small concentrations. The early experiments seemed to make no sense: Heparin blocked coagulation by 10^{-1} to 10^{-3} concentrations of endotoxin involving 10 µg or more of endotoxin, but had no effect at all on reaction mixtures containing nanogram concentrations of endotoxin.

We then realized that the high concentrations of endotoxin proceeded to completion within 10–20 minutes, but the low dose (i.e., 1–5 ng of endotoxin) required a considerably longer time. We also noted that heparin occasionally caused turbidity reactions in normal whole *Limulus* extract, which suggested that there might be irrelevant, nonspecific basic proteins in the *Limulus* extract that might themselves be combining with heparin and taking it out of the game. Then we capitalized on Levin and Bang's (6) observation that two distinct stages are involved in *Limulus* clotting and tested heparin against the second stage, i.e., the interaction between the thermolabile "enzyme" and the heat-stable coagulable protein. The results were much more reasonable and nonparadoxical. When we titrated heparin against the endotoxin-activated enzyme and the coagulable protein, we found that very small concentrations of heparin, down to 0.1 U/ml, were now completely protective. Note that this is not protection against the action of endotoxin itself; it is protection against the clotting reaction caused by the enzyme that is activated by endotoxin.

These concentrations of heparin are small enough to be meaningful and suggest that there may be, in fact, a connection between the mechanism of blood coagulation in higher animals and the clotting of *Limulus* lysate in the horseshoe crab. It is, of course, a long step from here to the hemorrhagic necrosis of vertebrate tumors which follows the iv injection of endotoxin, but perhaps the distance is not too great.

One is led to wonder whether there may still exist in vertebrates, higher vertebrates, even us, primitive precursors of the clottable protein of *Limulus* still present, maybe in the fluids bathing certain tumors or produced by them, maybe coming away from the endothelial cells of the special vessels feeding tumors. Clotting of such a material might be hard to detect in the cascading events of the normal coagulation mechanism. Solid material like this might be written off as fibrinoid or even mistaken as a badly formed constituent of fibrin. I suggest that we would do well to look hard for this kind of material, especially in lysates of neoplastic cells at the same time that the complexities of the *Limulus* reaction itself, especially the various inhibitors of this reaction that are known to exist in serum and tissue extracts, are being determined. It could turn out that the *Limulus* gel has a counterpart in mammalian pathology and that this might provide a new candidate mechanism for the seemingly insoluble problem of endotoxin in experimental pathology, e.g., a nonimmunologic one.

There is one other potential experimental model for the selective destruction of tissues in the field of marine biology, which ought to be looked into by people interested in tumor regression, although it has nothing at all to do with either immunology or endotoxin, or for that matter, with blood supply. I introduce it here simply because it seems interesting and may have some relation to the phenomenon, described by the Hellströms, of antagonistic reactions between nonimmunologic cell populations.

This is the phenomenon described several years ago by Jacques Theodor (7), at the Banyul Marine Biology Laboratory. Theodor has long been interested in the capacity of primitive, nonimmunologic animals to recognize "self" and "not-self" with accuracy. He used the arborescent coelenterate *Gorgonacea* as his experimental model. In this creature, segments of branches from one individual can readily be grafted to other regions, but grafts from other gorgonians are promptly rejected. When two gorgonians are fixed to each other, side by side, they regularly separate from each other.

In the course of this work, Theodor discovered a remarkable phenomenon that may shed light on the mechanisms by which one population of living cells are destroyed by a neighboring, foreign population. He found that when two fragments of gorgonian tissue were placed side by side in tissue culture, the larger fragment always caused total destruction of the smaller one. The small explant

simply vanished, apparently by cytolysis. However, it was not true that the larger tissue actually lysed the smaller one. On the contrary, the small fragment simply dissolved itself. Somehow, presumably on a signal conveyed by opposition of the membranes of the two explants, the smaller one set in operation its own mechanism for autolysis. When the small fragment, the target, was pretreated with dactinomycin or puromycin, no cytolysis occurred, but treatment of the larger, "killer" explant had no inhibitory effect. The phenomenon is autodestruction, performed after a cell population of a certain size (and perhaps possessing a certain, determinative rate of growth) receives a signal to destroy itself. It is not aggression as we usually interpret the antagonism between cell populations; the target population, in this case the smaller one, simply bows out.

My real purpose in bringing this rather arcane phenomenon into the present discussion is to illustrate the extremely broad area of basic biology that is relevant to the cancer problem. It might turn out that a better understanding of what actually goes on in Theodor's gorgonian explants, or for that matter in Cooper's earthworms which appear to be fully qualified to reject homografts of tissues from other earthworms (8), could be as useful for an understanding of spontaneous regression of human cancer as many of the more straightforward immunologic models with which most of us are concerned these days. Behind modern immunology, somewhat shadowed by its vast, complex, and sophisticated new structure, there stands a primitive, powerful defense apparatus, and we are not close to an understanding of what this is or how it works. We cannot safely assume that it has vanished from our level of biology, or, if it exists in us, that it simply rests there as a fossil. Perhaps, some of the mechanisms that operate so conspicuously in modern immunology are there as embellishments, adornments, like brightly colored feathers, and it is the old mechanism, whatever it is, that we need for an understanding of cancer.

REFERENCES

- (1) THOMAS L: Discussion of paper by P. B. Medawar. In *Cellular and Humoral Aspects of the Hypersensitive States* (Lawrence HS, ed.). New York, Hoeber-Harper, 1959, pp 529-530
- (2) BEER AE, BILLINGHAM RE: The embryo as a transplant. *Sci Am* 230:36-46, 1974
- (3) THOMAS L, DOUGLAS GW, CARR MC: The continual migration of syncytial trophoblasts from the fetal placenta in the maternal circulation. *Trans Assoc Am Physicians* 72:140-148, 1959
- (4) BANG FB: A bacterial disease of *Limulus polyphemus*. *Bull Johns Hopkins Hosp* 98:325-351, 1956
- (5) THOMAS L, GOOD RA: Studies on the generalized Schwartzman reaction. *J Exp Med* 96:605-624, 1952
- (6) LEVIN R, BANG FB: Clottable protein in *Limulus*. *Thromb Diath Haemorrh* 19:186-197, 1968
- (7) THEODOR J: Distinction between "self" and "not-self" in lower invertebrates. *Nature* 227:690-692, 1970
- (8) COOPER EL: Chronic allograft rejection in *Lumbricus terrestris*. *J Exp Zool* 171:69-74, 1969

Summary of Our Recent Studies on the Assessment of Cell-Mediated Cytotoxicity In Vitro¹

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SUMMARY—Experiments employing two new methods for the *in vitro* assessment of cell-mediated immunity against neoplastic target cells are indicated. Both methods offer the advantage of rapid and quantifiable determination of very early events in cell-cell confrontations. One technique measures the incorporation of precursors of protein, RNA, and DNA synthesis by neoplastic cells incubated with effector cells during the first hours of contact. It was found that exposure of BALB/c plasmacytoma (PCT) cells to splenocytes from PCT-sensitized donors failed to bring about inactivation of the target cells or their release of ⁵¹Cr during several hours of interaction. However, inhibition of ³H-thymidine incorporation was almost instantaneous and pronounced. This observation suggests that cytostatic events may transpire in cell-cell interactions in the absence, or before the onset, of lethal changes. The second method assays the specific adherence of ⁵¹Cr-labeled lymphoid cells from Rous sarcoma (RS)-bearing chickens to RS target cells. This immunoadherence test revealed preferential, specific reactivity in autochthonous as compared with allogeneic interactions, which indicated recognition of individual-specific tumor-associated antigens against a background of strongly expressed group antigenicities.—*Natl Cancer Inst Monogr* 44: 141, 1976.

To leave maximum time for Dr. Nossal's summation and for discussion (1), I would like to allude only briefly to several recent experimental findings in our laboratories; these findings focus on two immunologic aspects of host-tumor interactions that have not been stressed at these meetings so far.

1) In a series of *in vitro* studies of immunity against a plasmacytoma, Dr. Michael Steinitz and co-workers attempted to ascertain whether inhibition of target cell biosynthetic capacity could perhaps provide early and sensitive indications of cytotoxicity (2-4). We followed the effects of contact with splenocytes from tumor-bearing mice on the protein, RNA, and DNA synthetic capacities of BALB/c plasmacytoma target cells; the effector cells were of either syngeneic or allogeneic (C57BL) origin. In the allogeneic system, inhibition of DNA synthesis was virtually immediate upon contact with the effector cells, and stasis of RNA and protein synthesis became evident after a few hours. In the allogeneic system, one could, of course, also demonstrate gross damage to the cells in terms of liberation of ⁵¹Cr from the cell membrane as well as changes in the ability to exclude vital dyes. In the syngeneic interaction, however, we could detect no change in vital dye uptake ability and no liberation of labeled chromium, and yet there was, nonetheless, a virtually instantaneous inhibition of DNA synthesis. We might, in other words, have a model here of a strong cytostatic effect in the absence of, or preceding, lethal changes. I mention these observations because it is pertinent to emphasize in any immunologic analysis of host-tumor interactions the distinct possibility that there

exist cytostatic mechanisms that may be qualitatively, and not only quantitatively, distinct from cytolytic ones.

2) Dr. Mark Wainberg, Dr. Fanny Doljanski, and co-workers in our laboratories (5-7) did a series of studies with Rous sarcomas of chickens (5-7). In this model, we studied the specific attachment of sensitized lymphocytes to the sarcoma target cells, using a newly developed immunoadherence test based on quantification of the attachment of ⁵¹Cr-labeled effector cells to the target cells. In parallel, we determined the kinetics of the release of ⁵¹Cr from the targets brought about by effector cells and also by cytotoxic antibody. In all three test systems, there was a consistent superiority of autochthonous over allogeneic recognition. That is to say, lymphocytes from a tumor-bearing bird consistently, without a single exception, reacted more strongly against cells derived from their own tumor than against cells from any other Rous sarcoma of chickens. These findings suggest that there exists an individual-specific antigenicity of these neoplasms expressed despite the fact that in these tumors the common virus-associated antigenicity is a strong one. Therefore, one must again emphasize the need for studying host-tumor reactions in the autochthonous host, not only in terms of the lesser artificiality of the system but also in light of the eventuality that individual-specific, tumor-associated antigens may have a larger function in host-tumor associations than has hitherto been considered.

I thought it was pertinent to mention these two areas, as a kind of rounding out of the other aspects of immunologic interactions between host and parasite that have been discussed here.

REFERENCES

- (1) NOSSAL GJ: Spontaneous regression of cancer: Summary and profile for the future. *Natl Cancer Inst Monogr* 44:145-148, 1976
- (2) STEINITZ M, WEISS DW: Studies on the physiological manifestations of cell-mediated cytotoxicity. I. Early metabolic changes in mouse plasmacytoma cells exposed *in vitro* to sensitized allogeneic splenocytes. *Cell Immunol* 15:403-418, 1975
- (3) STEINITZ M, FEIGIS M, WEISS DW: Studies on the physiological manifestations of cell-mediated cytotoxicity. II. Inhibition of H3 thymidine incorporation by plasmacytoma cells exposed *in vitro* to sensitized splenocytes. *Cell Immunol* 17:181-192, 1975
- (4) ———: Studies on the physiological manifestations of cell-mediated cytotoxicity. III. Point of irreversibility of protein precursor uptake inhibition in plasmacytoma cells exposed *in vitro* to sensitized allogeneic splenocytes. Submitted for publication.
- (5) WAINBERG M, MARKSON Y, DOLJANSKI F, et al: Cellular immunity against Rous sarcomas of chickens. Preferential reactivity against autochthonous target cells as determined by lymphocyte adherence and cytotoxicity tests *in vitro*. *Proc Natl Acad Sci USA* 71:3565-3569, 1974
- (6) DOLJANSKI F, MARKSON Y, WAINBERG MA, et al: Cellular immunity to Rous sarcoma (RS)—preferential autochthonous interactions; and, application of the immunoadherence technique to mouse tumors. *Transplant Proc* 7 (Suppl 1): 519-523, 1975
- (7) WAINBERG MA, MARKSON Y, DOLJANSKI F, et al: Reactivity of serum from Rous sarcoma-bearing chickens with autochthonous and with allogeneic tumor cells: Preferential autochthonous recognition. *Int J Cancer* 15:984-994, 1975

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DISCUSSION

G. J. V. Nossal: I thought that I might start the discussion by asking you, Dr. Thomas, to extend your remarks about endotoxin and its curious membrane effects. We have been fascinated by the endotoxin molecule from two points of view recently. First of all, endotoxin causes the liberation of colony-stimulating factor, an entity capable of promoting the differentiation of marrow stem cells toward monocytoïd and granulocytoïd development. It does this extremely powerfully, within a few minutes of injection into animals, by mechanisms that appear to be nonimmunologic. Second, endotoxin, as we all know, acts as a kind of signal substance or coactivator for normally nonimmunogenic antigen molecules. Endotoxin clearly does something to the membrane of B lymphocytes, but again this is not strictly immunologic and certainly has nothing to do with the lymphocytes being able to recognize it specifically. So we are dealing with an agent that, putting it very broadly, is highly membrane active.

L. Thomas: It always seemed to me that, originally, endotoxin probably ought to have been given another name. It does not seem to be poisonous in the sense of doing real structural damage to anything. It behaves much more as though it were an almost universally important and significant biologic signal, and it goes about in the various hosts in which it is active, signaling a tremendous variety of totally unrelated cells and cell systems either to turn something on or off. In the generalized Schwartzman reaction, something rather like panic in a crowded theater sets in; an enormous number of otherwise well-designed and well-planned defense mechanisms are simultaneously turned on, and the animal is killed by this. The only clue I can think of that suggests a primary action of endotoxin is in the recent revelations in several laboratories that indomethacin and also aspirin will prevent the hemorrhagic and necrotizing properties of endotoxin, which suggests that an early step in the endotoxin reaction may be the turning on of prostaglandin synthesis and the elaboration of some as yet unidentified member of the prostaglandin series. I do not know whether anyone has tried using indomethacin or other prostaglandin synthetase inhibitors to interfere with the now nicely quantitated and measurable effects of endotoxin on cell populations, but it would be very important to have this information. I have no doubt any longer that lipid A is the operative part of endotoxin. It will do everything that the lipopolysaccharide will do.

D. Weiss: I would like to address a question to Dr. Alexander and Dr. Hellström. With regard to the blocking of effector cells by free tumor-surface antigen or antigen-antibody complexes, it may be true that whatever one attempts to do by way of specific or nonspecific immunization would be doomed to failure because one would have a vicious cycle in which, no matter how much one potentiates effector cell activity, one would be faced with a blocking situation. What would be the possibilities of removing from a tumor-bearing host his own lymphoid cells and permitting them to become resuscitated immunologically by incubation and/or repeated washing?

Would this manipulation be therapeutic after chemotherapy, radiation, or surgery?

K. E. Hellström: In my view, it depends on how strong the cell-mediated reactivity is and how high the blocking activities are.

Weiss: Dr. Alexander, would you care to comment on this?

P. Alexander: I think things are considerably more complex than has been indicated by these measurements. One expects to see excess antigen in the serum only when a large tumor is present. From a practical point of view, the challenge of cancer is not how to get rid of a large lump, because surgeons and radiotherapists do a rather good job of this. It is the question of how to deal with residual disease. The problem at the present time is that after surgical removal of the tumor, one no longer has any circulating antigen in the blood or the lymph and that one has plenty of cytotoxic cells; yet, unfortunately, we know that these primary treatments have a high failure rate and that disease often recurs. Therefore, I think anything we see in the serum is a late overflow effect and is not particularly relevant.

G. Klein: This may well be true with many situations, but certainly in the Burkitt patients whose tumors recur, antigen is detected in remission. I do not know how general that situation is, but I think one should look into it.

Alexander: Of course, the statement that you have antigen in these Burkitt patients in complete remission is rather a long extrapolation. The facts that one does know, which have been studied by others in detail, are that in melanoma, hypernephroma, and sarcoma, after surgical removal of the tumor, the circulating antigen levels fall right out and cytotoxic mononuclear cells can be detected.

G. Klein: I think the only system of human tumors in which we can really speak about antigens is the Burkitt system, because we are not postulating antigen from the blocking of lymphocytotoxicity but are measuring antigen directly.

Alexander: In other solid tumors, also, after surgical removal you get perfectly specific antibodies directed in the cross-reaction shown by Hellström; and you have, in fact, the complete mirror image in that when one has removed accessible tumor, one has antibody, which can be measured, and known antigen. Then the situation switches over. The identification relies on antibodies as well as on the secondary effect of inhibition of cytotoxic mononuclear cells.

Weiss: Perhaps we should open this discussion to questions from the floor.

P. Pattingle: This is a question to Dr. Klein. Infectious mononucleosis is assumed to be a self-limited, potentially malignant lymphoproliferative disorder caused by Epstein-Barr virus (EBV) and is assumed to be contained by a prompt immunologic protective response. What would you postulate for the mechanism of immunologic escape of a presumed EBV malignantly transformed clone of B cells?

G. Klein: I think at the moment you can entertain many different hypotheses. Of course, Burkitt himself has suggested that the curious geographic distribution of the

tumor is due to the transmission of the cofactor that promotes this very escape, and he suggested this cofactor is subclinical malaria; what is rather attractive about his suggestion is that subclinical malaria, big spleen disease, does indeed cause depression of T-cell immunity and that it stimulates at the same time the proliferation of B cells. It is a plausible hypothesis, but there is no evidence whatsoever except this sort of geographic distribution. One other thing comes up. There was a recent hypothesis in *The Lancet* that malaria is a B-cell mitogen. It was a hypothesis, but the malaria may prime B cells or more EBV target cells at a time when primary infection may occur. It is just an idea.

A. Bluming: Dr. Weiss, the nude mouse is a hairless mutant, as you know, that is born with the absence of thymic epithelium and therefore is a severe immunologic cripple. There are very nice pictures of the mouse growing chicken skin with feathers growing out of the skin, and I wonder if you or any other member of the panel can explain this phenomenon. When normal littermates of the nude mouse are painted with 7,12-dimethylbenz[a]anthracene, a strong chemical carcinogen, close to 100% develop skin tumors. When the same chemical is put on a nude mouse, practically no skin tumors develop. That is the opposite of what we would expect if the immune system was responsible for rejecting these tumors. What is more interesting, when these nude mice are reconstituted with thymic lymphocytes and then painted with dimethylbenz[a]anthracene, the incidence of tumors is exactly the same as it is in normal littermates. I wonder if you or any other member of the panel could offer some explanation for this very confusing finding?

G. Klein: Before you said the last part of the statement, I should have thought that probably the precursor cell was not present in the nude mouse. If thymic reconstitution works, then I have no explanation.

Alexander: I think you have touched on what to me, in the last 6 months, has been one of the most stimulating experimental investigations: this nude mouse situation. I think what the nude mouse has taught us is that the T-cell component of host immunologic defense is only a partial

component. It seems to be particularly important in the growth of foreign tissues at subcutaneous sites. It does not seem to be an important factor in preventing the dissemination of foreign tissue cells.

Weiss: Israelis, of course, have a penchant for answering a question with a question, so let me ask you in return, is it not conceivable that the presence of T cells will act to suppress, if one wants to view this in an immunologic framework? That is not saying very much more than that T cells may be in some circumstances the villain rather than the hero of the situation.

Bluming: Exactly. Thank you for that. I wonder if I may ask one more question. Dr. Klein, you mentioned Marek's disease and you mentioned the vaccine now used effectively to prevent the disease in fowls. When the herpesvirus of turkeys (HVT), which is the virus used for the vaccine, is given to fowls, they are immune to Marek's disease, but they do not eliminate the virus. The HVT replicates actively as does the Marek's agent; and the question is, what are your thoughts about how this vaccine is preventing the disease?

G. Klein: I think this is a very important experiment. Surely, it makes two points. One is that what you get in vaccination is not just antiviral immunity. I think it is very similar to the situation in which others have shown that, if you give one inoculation of simian virus 40 to hamsters, you get a certain incidence of tumors. If you give two, one to the newborn and the second before the tumors start appearing, then you prevent the tumors. So the virus is somehow alerting the immune system by giving more antigen to that situation. For Marek's disease, it will not do to give a second inoculation of virus; but this antigenic cross-reactive virus apparently does alert the immune system to prevent the outgrowth of the transformed cells, and this appears to be a dependent mechanism. Why the virus is spread, I think, is very easy to understand, because the virus matures only in the feathered foci when the cells start keratinizing. So no matter how much antiviral antibody serum circulates, the virus that is shed to the outer surface is not in contact with it.

Spontaneous Regression of Cancer: Summary and Profile for the Future¹

G. J. V. Nossal²

One unifying thread linking the parade of distinguished contributions to this symposium has been the fact that cancer can regress as well as grow. This is because, until very late in the disease, progression is balanced and influenced by host factors, of which the immunocyte army is among the most important. Known or unknown, spontaneous or induced changes in the balance of power between tumor growth drive and host defenses can and do occur, sometimes, though too rarely, eliminating the tumor in its entirety. As we struggle to integrate the many things we have learned, to unravel the complexities and balance the apparent contradictions, it may be wise to begin by going back to first principles. Let us identify the common ground, the facts on which we can all agree; then let us try to build up a dynamic theoretic framework from these facts; finally, let us ask what pointers for the future might emerge in the process.

CLINICAL DOCUMENTATION OF SPONTANEOUS REGRESSION

In our clinical documentation sessions, introduced with such engaging modesty by Dr. Cole, we agreed that spontaneous regression of advanced cancer was extremely rare, as an overall phenomenon occurring in less than one case per 1,000. We were reminded several times that 60% of the documented spontaneous remissions occurred in four rather special cancers, and that the cancers which account for 60% of overall mortality figured as only 12% of documented spontaneous remissions. For this reason, we took a particularly close look at the four examples for which spontaneous regression is slightly less rare, namely, hypernephroma, neuroblastoma, malignant melanoma, and chorionic carcinoma.

For neuroblastoma, as Dr. Evans explained to us, we face a very special situation, since two-thirds of the patients with cases of spontaneous regression are young, i.e., less than 6 months of age. Because both the antigenicity of the tumor and the presence of host killer lymphocytes are well documented, it seems tempting to ascribe spontaneous regression to the activities of a maturing immune system in these infant patients. Furthermore, neuroblastomas are unusual in that they may differentiate to a benign ganglioneuroma. One can surmise that such differentiation, apart from its intrinsic decrease in growth drive, could also involve the expression of new antigenic specificities that promote more vigorous host attack. Spontaneous regression of neuroblastoma at the microscopic level may be not at all rare, as "in situ" nests of neuroblastoma-like cells can be found in as many as 1% of adrenals of children (less than 3 months of age) who die from other causes.

This raises an extremely important point brought out by Dr. Milton Hyman from the floor, and reiterated in modi-

fied form by Drs. Nathanson, Ziegler, Lewison, and Alexander and others. It is the following: Complete regression of widespread metastatic disease is rare, but regression of small numbers of tumor cells at early stages of cancer may be almost commonplace. When a surgeon operates on Stage I breast cancer in a woman skilled at self-palpation of the breast and thus presenting early, he will achieve a better than 80% 5-year survival rate. Yet that tumor, to be diagnosed, must possess about 10^9 cells, and it is inconceivable that not a single one of those has escaped the confines of the lesion. This must mean that host defense mechanisms can frequently cope with a small tumor load.

Hydatidiform mole and chorionic carcinoma, as Dr. Hertz reminded us, present special features for students of spontaneous regression, because the invasive cells, originating from the embryonic trophoblast, are genetically foreign. They possess paternal *HL-A* genes, and I am a little more confident than was Dr. Hertz that the strong *HL-A* antigens that they express are a major feature in the relatively high regression rate. In all our experimental systems, *HL-A* antigens, the serologically recognizable cell-surface molecules by which organ grafts signal their foreignness to the host, are much stronger antigens than the relatively weak tumor-associated transplantation antigen(s) (TATA) of syngeneic systems. This suggests a link between three situations that we discussed as showing more frequent regression: chorionic carcinoma; maternal cancers transmitted to fetuses, as reviewed by Dr. Stephenson; and tumors accidentally transplanted with organ allografts that may regress when the recipient is taken off immunosuppression. In each case, the immune system has strong histocompatibility antigens on which to work, as well as any TATA that the tumors may possess.

Dr. Nathanson gave us an interesting dissection of the third special tumor, malignant melanoma. He showed us how similar the macroscopic and microscopic changes of spontaneous cure of the benign "halo nevus" were to the immunologically induced regressions in malignant melanoma, and to the relatively common spontaneous pigment changes and minor regressive episodes in malignant melanoma. While the extensive lymphocyte and macrophage activity in his sections certainly suggested intense immunologic activity, he warned us that hormonal factors should not be forgotten.

For hypernephroma, Dr. Shirmer reminded us that a proportion of the alleged spontaneous regressions followed after removal of the primary tumor, which, as we shall see, fits perfectly into an immunologic framework for spontaneous regression. Still we appreciated his note of caution about the inadequate documentation of many so-called regressing lung metastases.

Of the many other tumors that we heard about, Burkitt's lymphoma is probably most fascinating, and Drs. Ziegler and George Klein gave us up-to-date insights. Dr. Ziegler revealed some slightly disappointing news; although BCG treatment improved T-lymphocyte function, as judged by skin test reactivity, it did not alter

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patient prognosis, a conclusion which reinforces the gap between our present in vivo and in vitro testing methods and the knowledge that we wish to have about "real life" antitumor defenses.

From the other tumors we studied, the only links for spontaneous regression that we could find were: An overall tendency toward youth; a broad feeling that stirring up host defenses with infections, both bacterial and viral, might have helped in some cases; a concern that tumor tissue destruction and release by biopsy or irradiation might underlie some cases; and occasional hints that a changed hormonal status was at work. Dr. Lewison, Dr. Stephenson, and other speakers reminded us that literature search methods might underestimate the real frequency of spontaneous regression, and I would certainly ally myself with their pleas for a full report in the literature of each of these important natural events.

Dr. Southam (paper not published here) gave us a glimpse of a different type of spontaneous regression, that which can occur in autotransplanted human tumors, but which can be greatly augmented by the mixing of autologous white blood cells directly with the inoculated tumor cells. This initially surprising result is readily explained if we remember that there may be an access problem for lymphocytes which, having been appropriately stimulated, must nevertheless make direct contact with the tumor cell to kill it.

Dr. Bluming introduced us briefly to the new world of suppressor T cells and the major role of the T-cell system as a whole in controlling and "fine-tuning" the antibody response. His suggestion of B-cell cancer arising from antigenic stimulation unchecked by effective T-cell regulation is thought provoking. Yet I feel sure that Dr. Bluming would be the first to agree that the time has long since passed when we can regard the T cell as the universal friend and the B cell as the universal foe in cancer control. Also with Drs. Evans and Ziegler, he reminded us of two important things about chemotherapy in cancer control: 1) We must beware of overdoing things, lest our chemotherapy injure the lymphoid system irreparably. 2) We need to know much more about drugs that can specifically affect the T- versus the B-lymphocyte systems and their subpopulations.

We all agreed with our great absent friends, Drs. Doll and Berenblum, that cancer is a multistage phenomenon, but we were puzzled to define crisply progression from allegedly premalignant conditions to carcinoma in situ to frank malignancy. Drs. Firminger and Julian reminded us of Dr. Prehn's picturesque adaptation of Dr. Burnet's phrase, *clonal selection*, to describe aspects of this progression. Among populations of premalignant cells, all responding to a virus or carcinogen, a particular genetic variant, a single cell, may selectively grow and display greater malignancy, conceivably with changed antigenicity. The sequence may be repeated often, with progressively greater tumor cell autonomy. This capacity for mutation and selection in tumor cell populations is the largest single barrier to optimism for cure of advanced cancer by immunomanipulation. The risk of emergence of immunoresistant or antigenically mutant clones seems real.

SURGERY, RADIOTHERAPY, AND PSYCHOLOGICAL FACTORS

In this session, Dr. Ketcham (1) reemphasized for us that substantial regression could occur following inadequate surgery, and, on the whole, favored a changed immunologic balance of power as a plausible cause. He, too, gave some

credence to the potential importance of nonspecific stimulation of the reticuloendothelial system by infection and of frank or neurogenically induced hormonal changes. Dr. Hellman (paper not published here) was more reserved about the role of immunology. Both he and Dr. Pierce reminded us of a very important consideration, namely that the great majority of cells in most human tumors are *not* in cell cycle. There is a certain degree of maturation and of cell loss through what we might term natural death. After irradiation, regression seems chiefly due to a continuation of this spontaneous tumor cell loss not balanced by the activity of dividing cells, which have been destroyed, and there is thus no need to postulate accelerated immunologic destruction in the standard postirradiation situation. It is not excluded, of course, that the latter process could sometimes be operative. Hellman and Pierce both stressed that tumor size could be influenced by exquisitely small changes in the proportion of dividing cells; e.g., through processes of differentiation or dedifferentiation. Thus there are mechanisms of control far more subtle than numeric killing by antitumor lymphocytes, but our understanding of these is limited.

Dr. Mastrovito (paper not published here) in his important and very conservative talk, reminded us that cancer prevention and treatment must focus on the whole person and perhaps the whole society. I can only echo his view that we must take seriously the possibility that the higher cerebral centers can influence the incidence and progression of cancer, but that our most meticulous effort must go into the construction of *controlled* investigations, prospective if possible, if this field is to be integrated into the mainstream of cancer research.

AGREED FACTS IN IMMUNE RESPONSES TO TUMORS

We gave our most detailed attention to the host immune response, and it is this aspect which I can most fruitfully analyze in detail. To approach the problem, we must first give up some preconceptions about the immune response that we have from our days as medical students. Our paradigm of an immune response then was the single injection of a strong, particulate antigen. After a short latent period, this initiated a cascading, exponentially increasing proliferation of immunocytes, which formed large amounts of antibody. Our thinking about infections was dominated by diseases like smallpox, or diphtheria, when the immune system either "won," leaving the patient cured and immune, or "lost," resulting in death of the patient. We paid little attention to some model antigens which were poor immunogens or to host-parasite relationships in which chronic infection coexisted with modest or minimal immune responses. Few of us stopped to wonder, for example, why the levels of serum antibody against gut commensals, such as *Escherichia coli*, are uniformly quite low. We thought in terms of an avalanche, an almost all-or-none elimination of foreignness; but in tumor immunology, the analogy lets us down. Rather, we face an ecologic puzzle, in which the inherent proliferative drive of the tumor is impeded, but only partially and diminishingly, by a strictly limited immune response.

Fortunately, we have learned much at this meeting, and we had minimal conflict on the following five points:

1. Many experimental animal and spontaneous human tumors possess molecules on their surface which the host can recognize as antigen. These are especially evident in virally induced cancers. The categorization and quantitation of tumor antigens in man is only just beginning, though it is already clear that the induction or un-

masking of fetal antigens is particularly important, as are antigens specific for tumor tissue type.

2. The host can and does mount an immune attack against some of these antigens. The induction, nature, and intensity of this attack depends on many variables but can be manipulated intentionally by the clinician, though with results that are not always predictable.

3. Frequently, tumor growth activates all three major cell types of the immune system, namely the T lymphocyte, B lymphocyte, and macrophage. In vitro systems exist that can document the capacity of each type of cell to participate in tumor cell killing. The T lymphocyte can kill when the specific receptors of tumor-activated T lymphocytes meet and unite firmly with the antigenic determinants on the tumor cell. The B lymphocyte can kill more indirectly. It produces antibody, which can promote complement-mediated lysis or can, at low concentration, render the tumor cell susceptible to killing by a nonimmune lymphocyte with Fc receptors. The macrophage killing process, as Evans and Alexander and also Mackaness have shown us, involves the activation of the cell into an essentially nonspecific cytotoxicity, though the initiation of the activation depends on strictly specific events, probably largely the release of lymphokines from antigenically stimulated T cells. This effect on "innocent bystanders" is probably of special importance in the Edmund Klein experiments. Unfortunately, we cannot yet agree as to which of these killing methods is most relevant to the in vivo problems of visceral cancer.

4. All these potentially helpful killing possibilities can be rendered inoperative in vivo by plausible and well-documented obstructions. With solid tumors, there is a clear problem of access for the killer cell. Also, the lymphocyte receptors may become blocked before reaching the tumor site either by soluble, monomeric tumor antigen shed from the surface of the tumor cell during its normal metabolism, or, much more efficiently, by multivalent antigen-antibody complexes in the zone of slight antigen excess. We must recognize a twofold risk here. First, there is a potentially reversible blockade of the actual, activated, fully differentiated killer cell. This, having emerged from the antigen-driven clonal proliferation of its specific, resting precursor, is now prevented from performing its executive functions. It is locked in a cage of adherent antigen, rendered operationally multivalent by antibody. Secondly, there is an even graver risk. Under appropriate circumstances, both soluble monomeric antigens and highly multivalent antigens can cause irreversible tolerance induction among resting specific T and B cells, thus cutting off the potential immune avalanche at its very source. The larger the tumor load, the more real this possibility.

5. Though in specialized situations, such as the skin tumors or metastases described by Dr. Edmund Klein, the immune system can apparently destroy considerable amounts of tumor tissue. Everything we know from in vitro systems suggests that the killer potential of a given lymphocyte is finite, and that thus the rejection of a tumor depends on the relative numbers of killer cells to tumor cells. This indicates that intentional immunotherapy should be contemplated early rather than late in the natural history of the disease, and at a time when the target cell number has been reduced by surgery, chemotherapy, or radiation.

IMMUNOLOGIC EVENTS DURING PROGRESSION OF TUMORS

With this background of agreement, we come to our second task, namely to sketch out the likely immunologic scenario as a tumor arises and grows in a spontaneous human situation. The first question we must ask is one of antigen availability. For those cases in which the tumor arises because of some genetic change in a single cell, the problem initially is considerable, and one might assume that the premalignant clone would have to reach a reasonable size before delivering a readable antigenic signal. There would be other cases such as the one speculatively proposed by George Klein for Epstein-Barr virus-associated lymphoid cancers in which large numbers of virally transformed cells contain new antigens and the immune attack thus kills most of the potentially malignant cells, cancer arising only if one member "sneaks through." Equally, it is possible that in some chemically induced tumors, large numbers of cells are altered and expressed that change as an antigenic change, with only one of these altered cells eventually turning malignant through a further genetic event. In either of these two cases, the immunologic battle is joined

before malignancy supervenes. The reality of this possibility is underscored by a recent finding from our own group, albeit with an antigenic molecule that does not act as a TATA. It was found that a substantial proportion of chronic smokers 60 or more years old had high circulating levels of carcinoembryonic antigen, presumably similar to levels possessed by sufferers of bronchogenic carcinoma.

Whether the tumor mass is large or small, it is axiomatic that while a tumor grows, the host lymphoid system is exposed to progressively increasing levels of TATA reaching it in a variety of molecular forms. TATA molecules shed as soluble monomeric proteins from the tumor cell surface present an interesting problem. In many cases, they may closely resemble some "self" constituent, thus not presenting the kind of mosaic of antigenic determinants that optimally stimulates the cooperating T and B lymphocyte systems. The possibility of tolerance induction is very real. Secondly, fragments of tumor cell membranes and dying tumor cells could reach the draining lymph nodes. These have a better chance of being immunogenic, though in very high concentration or when complexed with antibody, they, too, could tolerize. Thirdly, occasional events of immune induction could occur when reactive lymphocytes actually encounter tumor cells as each wanders through the tissues. Broadly speaking, for most tumors some immunization should result during the earlier stages of growth. As effector cells are formed, various feedback loops will come into operation. Antibody will be formed, with the attendant possibilities of antibody-mediated tolerance and effector cell blockade. Killer cells will destroy tumor, causing pulsatile release of TATA, which again could be immunogenic or tolerogenic. The best chance the immune system has to destroy the tumor is at a relatively early (though not at the single cell) stage. If the inherent growth capacity of the tumor is sufficient to withstand the balance of immune activities engendered, and the tumor progresses, the odds in favor of immunologic nonreactivity increase. First, the continued absorption of antibody by the tumor cells lowers antibody levels; if antibody was originally in excess, it may fall to relative levels at which blockading or tolerance-inducing complexes, in slight antigen excess, will form. Secondly, increasingly high concentrations of antigen eventually blockade.

Conversely, if the tumor load is suddenly decreased, as after surgery or effective chemotherapy, this may significantly tilt the immunologic balance in favor of the host. TATA levels in extracellular fluids will fall, and, as many immunocytes continue to secrete antibody for some days in the absence of further antigenic stimulation, the extracellular fluids may move into antibody excess, allowing some cells to escape from the complex-mediated blockade. If residual tumor cells are not conquered then, the active immune response may decay for lack of antigen. At that stage, with antibody levels falling, the killing mediated by non-complement-dependent antibody, described by Holm and Perlmann, may be of special importance; so, indeed, may active immunotherapy. In the absence of total cure, the whole cycle may start again, though conceivably a relatively stable immune balance may be one of the factors behind the long latent periods in cancer progression.

THE FUTURE OF TUMOR IMMUNOLOGY

This broad scenario may seem a little trivial, but I have sketched it in some detail to permit me to address my third task, namely to develop a profile for the future. The immuno-oncologist must be the strategist and tactician in

the war between lymphocyte and cancer cell and, therefore, must know much more than he now does about the combatants. He must have good quantitative information about whether and to what degree his immunologic intervention has increased the activity of antitumor lymphocytes and lowered the tumor load. Therefore, two interdependent high technologies must be encouraged:

1. The techniques for quantitating antitumor lymphocyte activity must be improved. The Hellströms have set a fine lead, but we must go further. Colony inhibition is probably a compound test measuring both effector cells and the capacity to induce in vitro activation of precursor T and B cells. The technology of the future will need to separate these two aspects, probably by measurement of killing through short-term ^{51}Cr -release assays and of the precursor population through a formal, in vitro immunization protocol separated from a second-stage killer assay. Ideally, both T and B cell killing should be separately measured by use of cell separation methodology, and antibody levels should be continually monitored.
2. A molecular serology of tumor antigens will have to be built up. The classification and quantitation of tumor antigens in human sera will have to begin in earnest, following the sterling examples set by Gold, Morton, Alexander, and Baldwin. The beginning stages will be trying, as radioimmunoassays are the only feasible approach, and we will be titrating two unknowns, antibody and antigen. Nevertheless, recent history gives us cause for hope. When, only 15 years ago, Dausset and Payne began to identify human HL-A antigens, they faced the same puzzle; human sera with multiple specificities had to be fitted to human cells possessing at least four sets of major antigens. It was only the sheer willpower of the early groups in this field, e.g., Dausset, Payne, van Rood, Terasaki, Ceppellini, Bodmer, Morris, and their colleagues, that has today placed us in the luxurious position of being at the threshold of a complete understanding of this complex human polymorphism. With improved tissue culture technology to guide us toward banks of human tumor material, with gentler techniques of radioiodination to label either antigen or antibody, with, unfortunately, no dearth of sera (perhaps containing excess antibody at early stages or post surgery, and excess antigen at late stages), and with superb methods for separating proteins, we have the capacity to make rapid headway, if we have the will.

There are three possible pathways for active immunotherapy in the immediate future. First, we could press on with the magnificent and courageous empiricism of Mathé or Edmund Klein, painfully and over a long period building up sufficient clinical trial evidence to tell us which immunization procedure gives us what results in defined stages of particular tumors. Secondly, we could give up in despair at the complexity of the problem. Thirdly, we could roll up our sleeves and develop a molecular serology and lymphocyte biology in human cancer to outshine in its detail and precision the pathologic triumphs of the post-Virchow era. This would permit strict quantitative evaluation of every immunologic intervention. It would help not only the planning of immunotherapy but also the early serodiagnosis of cancer in high-risk groups and, perhaps, even accelerate the development of preventive measures. Dr. Holmes, in his talk here, summarized my position extremely well.

Of the many aspects of planning for this third pathway for the future which have engaged the attention of your country's experts, one seems to me worthy of special stress. The molecular serology of cancer will require banks of sequential sera taken from patients with well-documented cases, as in the experiments of Morton, Hellström, Weiss, and Alexander. Such serum banks could be regarded as a

valuable world resource, both for necessary standardization of assay procedures and, eventually, as a source of typing materials. Their disciplined collection and storage should begin now, and I would give Dr. Serpick my strongest support in his plea for centralized facilities to meet this need.

GENERAL PROSPECTS FOR CANCER RESEARCH

Many will criticize this profile as having placed too much emphasis on immunocytes and not enough on viruses. However, viral chemotherapy is not presently greatly encouraging and tumor-prophylactic viral vaccines seem currently relevant only to those, perhaps rather few, cancers triggered by horizontal infection. For vertically transmitted or oncogene-liberated viruses, immunologic tolerance remains a formidable obstacle. The most turbulent river to cross for cancer virus vaccines will be that of safety, in view of the relatively low case incidence rates and long latent periods of most cancers.

Our brief meeting has also tended to neglect genetics. In view of the great importance of immune regulatory genes in determining levels of responsiveness, and particularly of the *HL-A* complex, this field deserves our further support. Genetic analysis may well improve our understanding of risk factors. Time has not permitted us to delve into the fascinating worlds of cancer epidemiology or geographic pathology, nor of chemical carcinogenesis and the recent exciting potentials for prevention emerging from that field, yet any profile for the future must at least mention them. Nor do we wish to slight the endocrinologist, whose profile should be high as the field of hormone receptors on cancer cells develops. Of course, the three disciplines of surgery, chemotherapy, and radiation, the rocks on which our present considerable success in cancer rests, will each have their own noble evolution.

In summary, my profile for the future is not a pessimistic one. There are some who have criticized the high levels of expenditure on cancer research in this country, but let us look at this realistically. The health budget of the United States is \$100 billion, and if one computes that 10–20% of this is spent on cancer care, then the current cancer research program is still less than 5% of the gross total. The greatest need in this area is to maintain an even balance between fundamental and applied research, and to an outsider such as myself, it appears that you are doing a remarkable job in this regard.

Immunology will certainly not produce the magic panacea, the penicillin for cancer, nor does anyone at this symposium pretend that it will. However, given the disciplined approach that I have outlined and admitting that we are only a few halting steps along the road, I believe that immunology has a definite role that should make an honored contribution to the slow but relentless inroads into cancer mortality. If so, historians of the future will hail the pioneers who have graced this symposium, for they have made a brave beginning.

REFERENCE

- (1) SINDELAR WF, KETCHAM AS: Regression of cancer following surgery. *Natl Cancer Inst Monogr* 44:81–84, 1976

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